FP
317

HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ROBERT LARSSON

UNIVERSITY OF MICHIGAN
OCT 22 1954
SCIENCE LIBRARY

BAND XL HÄFT. 3—4

LUND 1954, BERLINGSKA BOKTRYCKERIET

HEREDITAS,

a periodical devoted to the publication of original research in genetics and cytology, is published by the MENDELIAN SOCIETY of Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. The current volume (XL) will contain about 500 pages, issued in four numbers.

Subscriptions should be sent to the Editor. Price *Thirty-five* (*35*) *Swedish Kronor per volume.* Postage free.

Back volumes. — The price of Vols. I—XII, XIX—XX, and XXIII is 15 Sw. Kr. each, of Vols. XXVI—XXXIII and XXXVII, 25 Sw. Kr. each, and of Vol. XXXIX, 35 Sw. Kr. Postage free.

(Vols. XIII, XIV, XV, XVI, XVII, XVIII, XXI, XXII, XXIV, XXV, XXXIV, XXXV, XXXVI, XXXVIII, and Suppl. Vol. 1949 are entirely out of print.)

ROBERT LARSSON
Editor of »Hereditas»
3 Adelgatan, LUND, SWEDEN.

REDAKTIONSKOMMITTÉ

PROFESSOR, FIL. DR. *ARNE MÜNTZING*
PROFESSOR, FIL. DR. *HERIBERT NILSSON*
PROFESSOR, FIL. DR. *GERT BONNIER*
PROFESSOR, MED. DR. *ERIK ESSEN-MÖLLER*

AN ANALYSIS OF HYBRID VIGOUR IN TETRAPLOID RYE

BY *ARNE MÜNTZING*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

IN breeding work with autotetraploid rye (*cf.* MÜNTZING, 1951) selection has been carried out within several different strains and also in crossing groups involving two or more strains. After intercrossing evidence of hybrid vigour and increased fertility was obtained several years ago. In order to obtain accurate data on this point artificial crosses between tetraploid strains of three different rye varieties, Steel, Petkus and King, were undertaken in 1951. The crosses were made in the three combinations possible (Steel×Petkus, Steel×King and Petkus×King; in the present paper the names Steel, Petkus and King always refer to tetraploid strains of these varieties), and in each cross in both reciprocal directions. The number of kernels obtained was sufficiently large to enable the establishment of isolation groups as well as a small field trial, in which parents and hybrids were compared. In this trial the seeds were sown in the holes of marker boards with one seed per hole, 5 cm. between the holes and 30 holes per row. In each cross there were 3 replications, each replication being represented by the parent strains (4 rows of each) and between them the two reciprocal hybrid combinations (2 rows of each). Thus, in each replication there was a total of 4 F_1 rows surrounded by 4 rows of each parent strain. To prevent border effects the whole trial was surrounded by tetraploid winter rye plants sown in spring and therefore not shooting. Germination and sprouting in the autumn were quite satisfactory and also the wintering and further development during the summer of 1952.

I. HYBRID VIGOUR IN YIELD PER PLOT.

The kernel yield per plot, expressed in grams per 2 rows, is given in Table 1. In this table the values of the parent strains have been halfed in order to become comparable to the two reciprocal F_1-combinations. As is evident from the table, the F_1 values are generally higher than those of the surrounding parent strains, and in all three crosses the average F_1 values surpass both parents. In the first cross

TABLE 1. *Kernel yield per plot (in grams per 2 rows).*

Material	Replication 1	2	3	Average
Steel	278	236	192	235,3
Steel×Petkus	281	195	246	240,7
Petkus×Steel	300	257	243	266,7
Petkus	196	183	201	193,3
King	152	163	164	159,7
King×Petkus	231	207	217	218,3
Petkus×King	194	239	249	227,3
Petkus	209	197	231	212,3
Steel	212	222	188	207,3
Steel×King	202	212	233	215,7
King×Steel	236	221	196	217,7
King	151	159	162	157,3

between Steel and Petkus the average value of the parents is 214,3 and that of F_1 253,7. The latter value is 18,4 per cent higher than the former one. In next cross, King×Petkus, the F_1 plots gave 19,8 per cent larger yield than parents, the average values of F_1 and parents being 186,0 and 222,8, respectively. In the third cross, between Steel and King, the F_1 superiority amounts to 18,9 per cent, the average values being 182,3 (parents) and 216,7 (F_1).

The significance of the differences between F_1 and parents was tested in two different ways with analyses of variance. The first way was to compare F_1 and P_m (parental average) in each cross separately and then to combine the data of all three crosses. Thus, for instance, in the first cross, Steel×Petkus, the P_m values of the three replications were 237,0, 209,5 and 196,5, the corresponding F_1 values being 290,5, 226,0 and 244,5. This will give the mean squares 2320,66 and 765,34 for variation between and within series, a v^2 value of 3,03 and a P intermediate between 0,2 and 0,05. Thus, in this cross the difference between F_1 and P_m is not significant.

In next cross, however (King×Petkus), the variation within series is smaller, the P_m values being 180,5, 180,0, 197,5 and the corresponding F_1 values 212,5, 223,0 and 233,0. This will result in the mean square 2035,04 for the variation between series and 102,17 for the variation within series. This gives a v^2 of 19,92 and a P intermediate between 0,05 and 0,01.

A still better significance was found in the third cross Steel×King, having the P_m values 181,5, 190,5, 175,0 and the F_1 values 219,0, 216,5,

214,5. The two mean squares will be 1768,16 and 32,84, respectively, giving a v^2 of 53,84 and a P intermediate between 0,01 and 0,001.

As all three crosses show similar differences between parents and F_1, it is well justified to combine the data by adding the sums of square for »between» and »within» and the corresponding degrees of freedom. This gave the following result:

Cross No.	»Between»	Degrees of freedom	»Within»	Degrees of freedom
1	2320,66	1	3061,34	4
2	2035,04	1	408,67	4
3	1768,16	1	131,34	4
Total	6123,86	3	3601,35	12
Mean squares:	2041,29		300.11	

This will result in a v^2 value of 6,80 and a P intermediate between 0,01 and 0,001. Thus, it may be concluded that in the three crosses studied the yield per plot in F_1 is significantly higher than the arithmetical mean of the parents.

As a safe establishment of this fact is a necessary condition for the detailed study to be described below, the difference between parents and F_1 was also tested in another way. Instead of using the P_m values for comparison with F_1, the values of the parent strains were kept separate and analyses of variance undertaken with the three series in question (2 parent series and one F_1 series). As there will be 2 degrees of freedom for the variation between series, this variation was subdivided into two components, one representing the difference between the two parents and the other one the difference between F_1 and the two parents. As usual, the mean square corresponding to the latter degree of freedom was divided by the mean square for the variation within series. In the cross Steel×Petkus the resulting P value was found to be intermediate between 0,2 and 0,05, whereas in the other two crosses it was intermediate between 0,01 and 0,001. Thus, with regard to crosses 1 and 3 this second statistical method gave the same P values as the previous method. With regard to cross No. 2 (King×Petkus), however, the P value was now one degree smaller than with the first method.

If the data obtained by the second method are combined by adding the sums of square and the degrees of freedom of the three crosses, a v^2 value of 6,38 will be obtained which with 3 degrees of freedom for »between» and 18 for »within» corresponds to a P which is again inter-

mediate between 0,01 and 0,001. Thus, the two statistical methods gave the same main result.

It should also be pointed out that the significance of the hybrid vigour observed may be demonstrated in a still simpler way. From the data given above it is evident that in all of the $3\times3=9$ comparisons between F_1 and the two parents the F_1 value (average of the two reciprocal combinations) is higher than the average of the parents. If there had not been any difference, the F_1 value would just as often have been below as above the parental average. Then the chance that the F_1 value would be higher in all nine cases without significant causes would evidently be $(^1/_2)^9=^1/_{512}=0{,}002$. This P value is again intermediate between 0,01 and 0,001.

II. THE SEPARATE FACTORS INCREASING YIELD IN F_1.

Kernel yield per plot is, indeed, a very complex character, and therefore it seemed desirable to try to find out at least some of the components leading to the increase in yield in F_1. Hence, a series of measurements were undertaken, the final results of which are given in Table 2. The values in this table represent F_1 values in per cent of the parental average, each figure being the mean value of the data from the three replications of each cross.

The general impression of the table is that in most cases F_1 surpasses the average of the parents, the percentage values generally exceeding 100. Different characters, however, behave differently in this respect. Thus, for instance, with regard to *kernel weight per plant* we find that the superiority of F_1 in the three crosses amounts to 9,5, 7,9 and 13,3 per cent, respectively. However, this superiority, though probably real, is not statistically significant. It was tested with an analysis of the kind described above as method No. 1, *i.e.* involving a comparison between F_1 and P_m. None of the separate crosses gives significant differences, and the combined P value for the whole material (Table 2, to the right) is intermediate between 0,2 and 0,05. Kernel weight per ear, on the contrary, gave a higher degree of significance, the first and third crosses giving P values intermediate between 0,05 and 0,01, whereas in the second cross P is intermediate between 0,01 and 0,001. The P value after combination of the data of the three crosses is smaller than 0,001 and, thus, highly significant.

In the same way the P values were calculated for the other measurements, excepting such characters where it is evident already from the

TABLE 2. *F_1 values in per cent of parental average.*

Character	Steel × Petkus		King × Petkus		Steel × King		Significance of combined data
	F_1 value	Significance	F_1 value	Significance	F_1 value	Significance	
Kernel weight per plot	118,4	$0{,}2 > P > 0{,}05$	119,8	$0{,}05 > P > 0{,}01$	118,9	$0{,}01 > P > 0{,}001$	$0{,}01 > P > 0{,}001$
» » » plant	109,5	—	107,9	$0{,}2 > P > 0{,}05$	113,3	$0{,}2 > P > 0{,}05$	$0{,}2 > P > 0{,}05$
» » » ear	110,3	$0{,}05 > P > 0{,}01$	112,4	$0{,}01 > P > 0{,}001$	107,2	$0{,}05 > P > 0{,}01$	$P > 0{,}001$
Number of kernels per plot	113,6	—	114,0	$0{,}05 > P > 0{,}01$	120,3	$P > 0{,}001$	$0{,}05 > P > 0{,}01$
1000-grain weight	103,3	—	105,5	—	98,7	—	—
Number of seedlings per plot	104,1	$0{,}05 > P > 0{,}01$	103,7	$0{,}2 > P > 0{,}05$	105,5	—	—
» » mature plants	106,2	$0{,}2 > P > 0{,}05$	110,9	$0{,}2 > P > 0{,}05$	106,0	—	$0{,}05 > P > 0{,}01$
» » ears per plot	107,1	—	106,3	$0{,}2 > P > 0{,}05$	112,8	$P > 0{,}001$	$0{,}2 > P > 0{,}05$
» » » » plant	100,4	—	96,0	—	105,2	—	—
» » flowers per ear	105,2	$0{,}01 > P > 0{,}001$	104,1	$0{,}05 > P > 0{,}01$	103,6	$0{,}2 > P > 0{,}05$	$0{,}01 > P > 0{,}001$
Per cent aborted flowers	98,5	—	98,7	—	108,9	—	—
Number of kernels per ear	106,6	$0{,}05 > P > 0{,}01$	107,1	$0{,}01 > P > 0{,}001$	106,4	$0{,}2 > P > 0{,}05$	$0{,}01 > P > 0{,}001$
Percentage of seed setting	101,3	—	102,8	$0{,}2 > P > 0{,}05$	105,0	$0{,}01 > P > 0{,}001$	$0{,}05 > P > 0{,}01$
Plant height	99,9	—	100,8	—	100,2	—	—

percentage values that there is no significant difference between F_1 and the parental average.

According to Table 2 the *number of seedlings* per plot, observed in the autumn a few weeks after sowing, is slightly higher in F_1 than in the parent, but only in one of the crosses, Steel×Petkus, does this difference reach the level of significance. In *number of mature plants* per plot the difference between F_1 and parents is more pronounced, indicating that the F_1 plants have been slightly superior in wintering ability or superior during the period of vegetative growth after wintering. The combined data give a P intermediate between 0,05 and 0,01.

F_1 is definitely superior in regard of *number of flowers per ear*, two of the crosses and the combined data showing a rather high degree of significance. The same is true of the *number of kernels* per ear, in regard of which the differences between F_1 and the parents are even still more marked. This is caused by the fact that also the *percentage of seed setting* is somewhat higher in F_1. The difference is slight, it is true, and only amounting to about 3 per cent, but the combined data of this character gave a P intermediate between 0,05 and 0,01.

Surprisingly enough the *number of ears per plant* was not higher in F_1 than in the parents. However, a probable tendency to an increased degree of tillering in F_1 will, in the present case, be counterbalanced by the presence of about 8 per cent more plants per plot in F_1 than in the parents. Thus, the greater crowding in the F_1 plots may tend to reduce the number of ears per plant. A negative coefficient of correlation ($r=-0{,}13$) was, indeed, obtained when correlating number of plants per plot and number of ears per plant but was not found to be significant.

Two other notable exceptions to the rule of F_1 superiority are represented by 1000-grain weight and plant height. Otherwise it is rather obvious that *the increased kernel yield, which for the three cross combinations amounts to about 19 per cent, is built up by a series of smaller superiorities of which the most clear ones are an increased number of flowers per ear, an increased percentage of seed setting and a higher number of mature plants per plot.*

As we have already seen, the higher number of mature F_1 plants is primarily caused by a superior ability to survive during the vegetative period of growth but probably also to a better sprouting ability, the number of seedlings per plot in the autumn being slightly higher in the F_1 plots.

Unfortunately the weight of the seeds sown was not determined, but there was no indication that the F_1 seeds were larger or of better

quality than the seeds of the parent strains, which were harvested on the same plots used for the artificial crosses. The average percentage of seed setting, when producing the F_1 seeds, was found to be 53, which is only a few per cent lower than the average percentage of seed setting in the parent strains. In the present trial the average percentage of seed setting was found to be 52,4 in tetraploid King rye, 61,5 in Steel and 61,6 in Petkus. Thus, the crossing had not resulted in a poor seed setting which would have tended to produce enlarged kernels.

III. RATIOS OF SUPERIORITY OR INFERIORITY IN VARIOUS CHARACTERS.

It was pointed out above that with regard to kernel yield per plot F_1 was superior to the parental average in all 9 replications representing the three crosses. The chance of such a fact occurring at random without significance would only be $(^1/_2)^9 = 0{,}002$.

The corresponding ratios and P values for all the other measurements undertaken are given in the right half of Table 3. Some of the characters have given 9 : 0 ratios (P=0,002), *viz.* number of flowers, number of kernels and kernel weight per ear. In some other cases the ratio is 8 : 1, indicating that in one case of nine F_1 has been inferior to the parental average, in the other eight cases superior. The P value of getting such an extreme distribution by mere chance is 0,02. This ratio

TABLE 3. *Ratios of superiority or inferiority in various characters.*

Character	F_1 compared with superior parent		F_1 compared with parental average	
	Ratio	P value	Ratio	P value
Kernel weight per plot	7 : 2	0,09	9 : 0	0,002
» » » plant	5 : 4	0,50	8 : 1	0,02
» » » ear	5 : 4	0,50	9 : 0	0,002
Number of kernels per plot	7 : 2	0,09	8 : 1	0,02
1000-grain weight	1 : 8	0,998	5 : 4	0,50
Number of seedlings per plot	7 : 2	0,09	8 : 1	0,02
» » mature plants	7 : 2	0,09	8 : 1	0,02
» » ears per plot	7 : 2	0,09	8 : 1	0,02
» » » » plant	3 : 6	0,91	4 : 5	0,75
» » flowers per ear	4 : 5	0,75	9 : 0	0,002
Per cent aborted flowers	6 : 3	0,25	4 : 5	0,75
Number of kernels per ear	4 : 5	0,75	9 : 0	0,002
Percentage of seed setting	2 : 7	0,98	8 : 1	0,02
Plant height	1 : 8	0,998	4 : 5	0,75

and P value were found in regard of the following characters: number of seedlings per plot, number of mature plants, number of ears per plot, number of kernels per plot, kernel weight per plant and percentage of seed setting. These characters form a group well separated from a group of other characters, having ratios of 5 : 4 or 4 : 5 and large P values. These characters are plant height, per cent aborted flowers, number of ears per plant and 1000-grain weight.

A comparison between these data in Table 3 and the figures in Table 2 is of interest. In all cases in which, according to Table 2, the P values were smaller than 0,05 we have also in Table 3 P values which are either 0,02 or 0,002. It should be observed, however, that in three cases (number of seedlings per plot, number of ears per plot and kernel weight per plant) the calculations represented by Table 3 gave rather significant P values (P=0,02), whereas the result of the analyses of variance for the same characters given in Table 2 was non-significant P values larger than 0,05.

The values in the left half of Table 3 indicate to what extent F_1 is superior to *both* parent strains and thus showing an obvious degree of heterosis. The ratios in this respect range from 7 : 2 to 1 : 8, and all the corresponding P values are equal to 0,09 or larger. Thus, in no character is F_1 with certainty superior to both parents. It should be observed, however, that for the characters showing a 7 : 2 ratio (F_1 being superior to both parents in 7 cases of 9), the odds are 91 : 9 that there is a real superiority. This was found to be true of the compound character kernel yield per plot as well as of some of its components, *viz.* number of seedlings, number of mature plants, number of ears and kernels per plot.

It is rather striking that in some characters in which F_1 is significantly superior to the parental average, it is by no means significantly superior to both parents. Such is, for instance, the case with regard to the number of flowers per ear, kernel weight per ear and percentage of seed setting.

IV. DISCUSSION.

In several species of autogamous plants hybrid vigour has been observed in F_1 combinations of different pure lines (*cf.* MÜNTZING, 1945; HAGBERG, 1952 a and b), but as is well known, the most striking increases in vigour are obtained in allogamous plant and animal species by the intercrossing of inbred lines. Similar increases have also been observed to occur in certain crosses between different allogamous populations. The cases of this kind first known are reported by ZIRKLE (1952).

According to him, BEAL in 1880 succeeded to increase the yield of corn by intercrossing two stocks of the same type of corn which had been grown a hundred miles apart for a number of years. The increase in yield is said to amount to 51 per cent, but this figure probably involves only one of the parental varieties in relation to F_1. SANBORN (1890) confirmed BEAL's result and reported that his own hybrid corn yielded in the ratio of 131 to 100 for his inbred. MORROW and GARDINER (1893 a and b) reported that in each of five different crosses investigated the yield from the cross was greater than the average from the parent varieties. According to them, it seems that crossbred corn gives larger yields at least for the first and second years after crossing than an average of the parent varieties. A very marked increase in plant height was observed by WEBBER (1900) in F_1 of the cross »Hickory King × Cuzco», the former being a native North-American variety, the latter a variety from Peru. According to EAST and JONES (1919), increases in yield of grain are frequently obtained when ordinary commercial varieties of maize are crossed. Rarely are the increases greater than 10 per cent, however, and even this is more commonly to be expected when varieties of somewhat different type are used; for example, flint and dent. COLLINS (1910) obtained especially large increments in yield by hybridizing types of corn from different geographical regions.

In rye the effects of intercrosses between different diploid varieties have been studied by HAGBERG (1952 c). He made a series of pair crosses between and within the summer rye varieties Petkus, Giant and Od. The F_1 plants showed no indication of heterosis in the length of the straw but only in tillering and, consequently, in weight of plant and yield of grain per plant. The material was too limited to allow calculations of the yield per plot. In all three F_1 combinations possible kernel yield per plant was found to be higher than in the best of the parent varieties. This superiority amounted to 17 per cent in the cross Giant × Od, to 12 per cent in Petkus × Od and to 2 per cent in Giant × Petkus. The degree of hybrid vigour in the latter cross is supposed to be low because Giant and Petkus are probably very closely related.

In the same paper HAGBERG reports that, in contrast to rye, intercrosses between eight local strains of red clover failed to reveal any significant differences between inter-strain and intra-strain crosses. HAGBERG assumes that this is due to the fact that the genetical variation within the clover strain is still very extensive, even in comparison with the differentiation between strains.

The data reported in the present paper show that hybrid vigour in

rye is not limited to diploid material but also occurs in F_1 combinations between tetraploid varieties. In the three F_1 combinations studied kernel yield in F_1 was found to be 18,4, 19,8 and 18,9 per cent higher than the average of the parents and 7,8, 4,9 and 4,5 per cent higher than in the best parent. This degree of hybrid vigour seems to be lower than in the crosses Petkus×Od and Giant×Od studied by HAGBERG but higher than in his cross Petkus×Giant.

One reason for a low degree of hybrid vigour in the present crosses is the fact that the three varieties involved are rather closely related, King rye representing a selection from Steel and Steel a selection from Petkus. However, there is another factor to consider which will operate in the opposite direction, *viz.* the fact that the tetraploid varieties are derived from a rather limited number of original diploid individuals. This is especially true of tetraploid Steel which represents a combination of the genes present in the nine diploid plants, from which this variety is derived (MÜNTZING, 1951, p. 18). In tetraploid King the number of original diploid individuals is somewhat higher (MÜNTZING, unpublished). In tetraploid Petkus, being produced in Germany, the corresponding number is unknown to me.

In contrast to the material of HAGBERG (1952 c), in which the superiority of the F_1 plants was due to an increased degree of tillering, the number of ears per plant in the tetraploid material was not higher in F_1 than in the parents. This difference between the diploid and tetraploid rye material may be only apparent, the tillering in the tetraploid F_1 plants being counterbalanced by a higher number of mature plants in the F_1 plots than in the parental plots. Furthermore, in HAGBERG's material the individual plants had better opportunities of tiller development than in the tetraploids, the distances between the plants being 10 cm. in the former material, 5 cm. in the latter one.

The main point of interest in the present paper is the fact that the increased kernel yield in F_1 is built up by a series of smaller superiorities, of which the most clear ones are an increased number of flowers per ear, an increased percentage of seed setting and a higher number of mature plants per plot. This is, indeed, to be expected with regard to such a complex character as kernel yield which is known to be conditioned by the combined effects of many genes. It is of interest, however, to demonstrate that not a single or a few but rather a series of different circumstances with more or less slight positive effects may combine to give a very marked final result.

This is probably a universal phenomenon and has been observed

more or less clearly in various species of plants and animals. Thus, for instance, a study of Table 2 in a paper on heterosis by SHULL (1952) reveals that in maize the difference in yield between inbred (A) and crossbred (C) material is dependent on number of stalks per plant, on number of grain rows per cob and probably also on plant height. Relatively moderate superiorities in these respects combine to give a very large superiority in yield per acre. Thus, if the number of stalks is 71 in the inbreds and 91,3 in the crossbreds, and the corresponding pairs of values are 12,6 and 16,9 for grain rows and 19,3 and 23,5 for plant height, this corresponds to a yield of 25,0 bushel per acre in the inbred material and as much as 63,5 bushels per acre in the crossbred group.

EAST and JONES (1919, p. 161) quote a similar example from a material of guinea-pigs studied by WRIGHT. Crossbred animals were distinctly superior to their inbred relatives in nearly all characters connected with vigour. In spite of the fact that their inbred mothers were small and somewhat deficient in vigour, a slightly larger per cent of crossbreds than of inbreds were born alive, and a distinctly larger per cent of those born alive were raised. They were somewhat heavier at birth in a given size of litter and gained in weight much more rapidly between birth and weaning. They matured earlier and produced larger litters and produced them more regularly than the inbreds.

Another similar example concerns fertility in *Drosophila melanogaster* (GOWEN, 1952). Lifetime egg productions show greater heterosis than any of the component factors which ultimately determine it. Length of egg laying period has 113 per cent heterosis, maximum egg production 154 per cent, and resistance to decline in vigour, as measured by egg production with advancing age, 120 per cent, while the over-all character lifetime egg yield has 203 per cent heterosis. Heterosis also in this case appears to be a consequence of the combined action of two or more groups of distinct and more elementary characters which, when jointly favourable, lead to generally high yields.

HAGBERG (1952 b) has found that in F_1 hybrids between pure lines in *Galeopsis* a favourable combination of the different growth rhythms of the parents is frequently obtained. To cover such cases he has coined the term *combination heterosis*. This indicates that F_1 combines from the parents two or more traits, each one favourable for the total vigour, without showing heterosis in any one of them separately. This evidently applies very well to the present material of tetraploid rye and also to the other instances mentioned.

It should be observed that with regard to the use of the term

heterosis HAGBERG (1952 a) adopts the view that F_1 should be compared to the superior parent, and that true heterosis or luxuriance only occurs when F_1 is superior to the best of the parent lines. In my opinion the frequent occurrence of combination heterosis, in which a series of slightly positive traits combine to give a marked end result, speaks against the biological validity of a heterosis concept defined as superiority of F_1 over the best of the parents. Evidently there is no essential difference between cases in which F_1 is superior to the arithmetical average of the parents and cases when F_1 happens to surpass also the superior parent. The latter alternative will happen more frequently when the parents have similar values in regard of the characters measured. If, on the contrary, there is a wide difference between the parents, it is much less likely that F_1 will surpass both parents. Nevertheless the same biological phenomenon is at work in both cases, a superiority of F_1 in various respects, which is most conveniently demonstrated by comparisons between F_1 and the average values of the parents.

SUMMARY.

(1) Tetraploid strains of the rye varieties Petkus, Steel and King were intercrossed and the resulting three F_1 combinations compared with the parents in a yield trial. Kernel yield per plot was significantly higher in F_1 than in the parents, the superiority of F_1 over the parental average ranging from 18,4 to 19,8 per cent.

(2) Detailed data were gathered for 13 characters, and it was found that most of them contribute to the hybrid vigour in kernel yield. This is especially true of the number of flowers per ear, the percentage of seed setting and the number of mature plants per plot.

(3) Other cases of combination heterosis are discussed, and it is pointed out that there is no essential difference between cases in which F_1 is superior to the average of the parents and cases where F_1 happens to surpass also the superior parent.

LITERATURE CITED.

1. BEAL, W. J. 1880. Indian corn. — Rept. Michigan State Board Agric., Vol. 19, pp. 279—289.
2. COLLINS, G. N. 1910. Increased yields of corn from hybrid seed. — Year-book U. S. Dept. Agr., pp. 319—328.

3. EAST, M. E. and JONES, D. F. 1919. Inbreeding and outbreeding. — Philadelphia and London, pp. 1—285.
4. GOWEN, J. W. 1952. Hybrid vigour in *Drosophila*. — Heterosis, Ames, Iowa, 1952, pp. 474—493.
5. HAGBERG, A. 1952 a. Heterosis in F_1 combinations in *Galeopsis*. I. — Hereditas XXXVIII, pp. 33—82.
6. — 1952 b. Heterosis in F_1 combinations in *Galeopsis*. II. — Hereditas XXXVIII, pp. 221—245.
7. — 1952 c. Heterosis in some crosses between populations of rye and red clover. — Hereditas XXXVIII, pp. 505—510.
8. MORROW, G. E. and GARDINER, F. D. 1893 a. Field experiments with corn. — Illinois Agric. Expt. Sta. Bull. 2, pp. 173—203.
9. — and — 1893 b. Field experiments with corn. — Illinois Agric. Expt. Sta. Bull. 2, pp. 333—359.
10. MÜNTZING, A. 1945. Hybrid vigour in crosses between pure lines of *Galeopsis Tetrahit*. — Hereditas XXXI, pp. 291—398.
11. — 1951. Cyto-genetic properties and practical value of tetraploid rye. — Hereditas XXXVII, pp. 17—84.
12. SANBORN, J. W. 1890. Indian corn. — Rept. Maine Dept. Agric., Vol. 33, pp. 54—121.
13. SHULL, G. H. 1952. Beginnings of heterosis concept. — Heterosis, Ames, Iowa, 1952, pp. 14—48.
14. WEBBER, H. J. 1900. Xenia or the immediate effect of pollen in maize. — U. S. Dept. Agric. Div. Veg. Phys. Bull. 22.
15. ZIRKLE, C. 1952. Early ideas on inbreeding and crossbreeding. — Heterosis. Ames, Iowa, 1952, pp. 1—13.

CONTENTS.

I. Hybrid vigour in yield per plot 265
II. The separate factors increasing yield in F_1 268
III. Ratios of superiority or inferiority in various characters 271
IV. Discussion 272
Summary 276
Literature cited 276

STUDIES ON SELF-STERILITY IN RYE, SECALE CEREALE L.

BY *ARNE LUNDQVIST*
INSTITUTE OF GENETICS, LUND, SWEDEN

PRONOUNCED self-sterility has long been a well-known phenomenon in rye. As early as in 1877 RIMPAU (quoted from ULRICH, 1902) proved the predominance of self-sterile individuals in populations of rye. Since that time, the knowledge of occurrence and inheritance of this self-sterility has been extended by several investigations (*e. g.*, HERIBERT NILSSON, 1916; BREWBAKER, 1926; PETERSON, 1934; HERIBERT NILSSON, 1953). Different degrees of self-sterility were proved within and between different varieties of rye, and these differences seemed to be strongly conditioned by genetic factors. The latter two authors suggest that self-sterility in rye might be controlled by oppositional alleles of the well-known Personate type. However, a strict genetic analysis of the mechanism of self-sterility, has not as yet been accomplished owing to serious practical difficulties, the material being annual with a concentrated flowering period and few inflorescences. In connection with an investigation of the effects of inbreeding in rye, the need of such an analysis was realized by the present writer.

MATERIAL AND METHODS.

The material studied belongs to the Swedish diploid commercial variety »Stålråg» (Steel-rye). This is a very highbred variety derived in 1911 as a single plant selection from the Swedish »Stjärnråg» (Star-rye), which, in its turn, is derived from the famous Petkus-rye (LJUNG, 1921). On selfing after parchment bagging, seed setting in Steel-rye has averaged 2,5—3,5 %, and about 90 % of the plants have been below the limit of 5 % seed setting (MÜNTZING, 1943; LUNDQVIST, 1947).

22 plants taken from an élite population, were cloned in the autumn of 1951. The majority of these clone plants were exposed to frost, and tillered next summer. After selfings fairly large S_1 progenies were obtained from a number of these clones. By means of repeated cloning and delayed cold treatment most of the remaining clone plants were

TABLE 1. *Material analysed. Results of selfings, backcrosses in both directions, and intercrosses.*

(Self- — = self-incompatible; Self- + = self-compatible; + = compatible matings.)

a Parents			*b* S_1 generations				*c* Mating categories								
Parent clone number	% seed setting on selfing		S_1 generation		% seed setting on selfing		$S_1 \times P$			$P \times S_1$			$S_1 \times S_1$		
							Number		%	Number		%	Number		%
	1952	1953	Kernels sown	Mature plants	Self- — 1953	Self- + 1953	total	+	+	total	+	+	total	+	+
1	2,65	0,00	20	10	1,69		10	9	90				8	6	75
						(1 plant)									
2	2,05		20	13	0,28	77,14							7	5	71
3	0,31	0,00	18	10	0,10		5	2	40				21	8	38
4	1,64	0,76	20	13	0,38		13	10	77				10	2	20
5	0,64	0,00	18	13	1,52		13	10	77	7	—	—	12	10	83
6	1,48	1,28	20	7	1,60		6	4	67	1	—	—	7	4	57
9	1,73		18	10	0,21								16	13	81
13	0,60		17	11	0,15								18	9	50
14	1,32		4	4	2,47								5	3	60
						(7 plants)									
15	1,68	1,49	20	13	3,45	49,27	13	9	69	1	—	—			
16	1,14	5,23	20	14	0,81		14	10	71	8	—	—	8	7	88
						(1 plant)									
17	0,66	0,28	8	8	0,00	50,00	8	6	75	5	—	—	4	2	50
18	0,22	0,14	2	2	0,00		2	2	100				1	1	100
20	5,11	5,49	20	12	1,87		12	10	83	8	—	—	4	2	50
21	11,31	13,37	20	15	5,65		15	13	87	8	—	—	6	3	50
Total			245	155			111	85		38	—		127	75	
%	2,17			63,3	1,35			77			0			59	

forced to a delayed tillering in the summer of 1953. In these cases it was possible to test the S_1 progenies on parental plants.

Following a delayed sowing, caused by a retarded harvest of the material, the S_1 progenies did not winter well. Practically all seed sown germinated, but not more than about 60 % of the original material was available for the experiments in the summer of 1953 (Table 1 *b*). This reduction, however, is not likely to have seriously influenced the representativeness of the material. The plants were kept in boxes outdoors, and only immediately before flowering were they transferred into the greenhouse, as were the parental clone plants also, previously grown in beds.

Within the material available, S_1 plants were selfed, intercrossed, and, if possible, reciprocally backcrossed to their parents. All the crossings were made in the greenhouse, and included emasculation and manual pollination with collected pollen. No complete security was obtained, however, against illegitimate pollination during these manipulations as another material of flowering rye was also kept in the greenhouse, but considering the undisturbed air conditions of the greenhouse, the risk of contamination was probably not significant. The pollen was examined under a microscope before the pollinations to control that it contained morphologically well developed pollen grains. The interval between emasculation and pollination varied between 1 and 14 days, and commonly numbered 4—8 days. Data from ears with an interval of more than 15 days were excluded, as a detailed study of the seed setting in different parts of the ears revealed that, in these cases, their ability to conceive had declined. The classification of mating results was not based on the absolute values of seed setting, but made allowance for the age of the ear at the time of pollination, and the distribution of seed setting in the ear. Concentration of seed setting to the uppermost and, especially, to the lowermost parts of the ear, was taken as an indication of decreasing ability to conceive.

EXPERIMENTAL RESULTS.

SELFINGS.

In most cases the parental clones could be selfed in two subsequent years, and the values of seed setting have been summarized in Table 1 *a*. For different practical reasons, 7 of the original 22 clones were discarded after the first year. Seed setting of the selfings of 1952 averaged 2,17 %, which is in good agreement with the values obtained in previous

more extensive selfings in Steel-rye (MÜNTZING, *l. c.*; LUNDQVIST, *l. c.*). Of special interest is the good agreement within different clones between the values of seed setting on selfing from the two years. The self-sterility of the plants is unimpaired by their longevity, and the different degrees of self-sterility are obviously only to a small extent conditioned by external influences.

Selfings of plants in the S_1 progenies (Table 1 *b*) give further support to the impression that the different degrees of self-sterility are clearly genetically conditioned. An analysis of the correlation between the values of the clone selfings in 1953 and the corresponding values from selfing in the S_1 progenies, will give a correlation coefficient $r = +0{,}77$ $(0{,}01 > P > 0{,}001)$. In some of the S_1 progenies, selfing also revealed a very distinct group of self-fertile plants. The average fertility on selfing of this category has been entered separately into Table 1 *b*.

CROSS-POLLINATIONS.

The self-fertile S_1 plants were compatible as male, both in backcrosses to their parent plants, and in intercrosses to their siblings. As female parent, on the other hand, they did not differ from the other plants in their average compatibility reaction. As they were considered to constitute a separate problem their mating results as males will not be treated in this connection. It should be pointed out, however, that LEWIS (1949), discussing the question of mutations in incompatibility alleles, presented arguments which may very well be applicable to the present cases, too.

The remaining results have been summarized in Table 1 *c* under three different headings: backcrosses in both directions, and intercrosses. For each separate parent-offspring group, the table presents within each category the total number of mating combinations tried, and the number and percentage of them classified as compatible.

Some regularities are quite apparent in Table 1 *c*. Backcrossed as male, the parental plants are predominantly — considering the whole material in 77 % of the cases — compatible with their S_1 plants. Backcrossed as female, on the other hand, they are completely incompatible. Though the restricted numbers of matings within the individual parent-offspring groups, will not permit a statistical evaluation of the similarities between different groups in their incompatibility reactions, these similarities are considered to be great enough to warrant, in the following, the individual values being summed up and treated as an entirety.

TABLE 2. *Frequencies of compatible and incompatible matings within S_1 progenies.*

(S_1 plants grouped according to their compatibility reactions as female with their parent plants.)

Parent clone number	*a* Compatible S_1 plants				*b* Incompatible S_1 plants			
	pollinating		pollinated		pollinating		pollinated	
	+	−	+	−	+	−	+	−
1	6	1	7	1	1	—		
3	2	4	2	1	2	3	2[1]	4
4	2	8	2	3			—	5
5	4	2	10	—	6	—	—	2
6	2	2	4	2	2	1		
16	7	—	7	—	—	1	—	1
17	1	2	2	1	1	—	—	1
18	1	—	1	—				
20	1	2	2	—	1	—	—	2
21	2	3	3	1	1	—	—	2
Total	28	24	40	9	14	5	2	17
%	54		82		74		11	

[1] Belong to the same plant.

Intercrossing within the S_1 progenies resulted in reciprocally compatible, incompatible, and one-way compatible mating combinations. In all, 59 % of the matings were compatible. Backcrossed as female to their parent plants, 78 % of the S_1 plants in question were compatible; the remaining 22 % were incompatible. Intercrossed as male parent to their siblings the former group were compatible in 54 %, as female parent in 82 % of their mating combinations (Table 2 *a*). The corresponding values for the backcross incompatible group, were 74 % and 11 %, respectively (Table 2 *b*). The similarity in the compatibility reactions between this latter group and their parental plants, is striking. Summarizing the results in Table 2, it might be stated that plants most successful as male parent are the more incompatible as female parent, and conversely.

GENETIC INTERPRETATION.

The clear-cut results from the reciprocal backcrosses together with the similarities in incompatibility reactions of parental plants and of

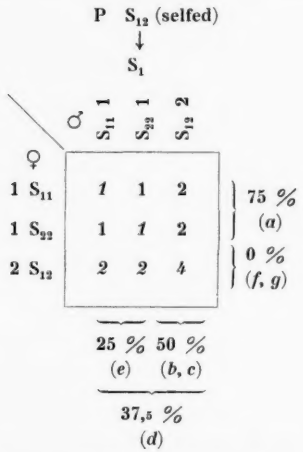
backcross incompatible S_1 plants, respectively, seem to indicate a relatively simple genetic mechanism controlling incompatibility in rye. The results, however, are not in accordance with those expected from the systems recognized as controlling self-incompatibility in homomorphic flowering plants — the Personate system and the Composite system, respectively — so far as their typical cases have been outlined in diploids. The main characteristic of the rye system is obviously the high frequency of compatible mating combinations, as compared to that following either of the two other incompatibility systems.

In both these systems the incompatibility is controlled by a multiallelic locus. When stylar tissue and anther tissue have one or both of the possible alleles in common, a pollination may be followed by an inhibited pollen tube growth. In the Personate system, however, the anther tissue will only indirectly, by means of the meiotical factor apportionment, be capable of determining the reaction of a certain pollen grain: the pollen reacts with a given stylar tissue completely according to its own genotype (EAST and MANGELSDORF, 1925). In the Composite system (HUGHES and BABCOCK, 1950; GERSTEL, 1950) the anther tissue will determine the incompatibility reactions of the pollen grain directly: with a given stylar tissue all pollen grains from a common source, will react alike, irrespective of the different genotypes they might have obtained at meiosis. Finally, the gene action of the stylar tissue is independent, in the Personate system at least: each allele of the style is capable of producing a complete incompatibility reaction with a pollen grain independent of its partner allele in the style. In the Composite system there might sometimes be some complications, resulting from dominance interactions between the alleles in the style, *e. g.* in *Cosmos*, and perhaps in *Parthenium* (CROWE, 1954).

Compared to a gametophytic pollen control, the sporophytic pollen control will involve an increased proportion of incompatible mating combinations. The parental plant will in the extreme case be reciprocally incompatible with its S_1 offspring. At the same time the frequency of compatible sib-matings within the S_1 offspring will at least equal that of the mating S_1 plants$\times$parental plant. The Composite system, even in modified form, will obviously not be applicable to the rye data.

The incompatibility relations under the control of a Personate incompatibility system, are illustrated in Scheme 1, and summarized in Table 3 *A*.

Table 3 *B* presents the values obtained from rye. The similar ten-



Scheme 1. Incompatibility relations within a parent-offspring group. One Personate incompatibility factor system assumed. (Combinations with no factor in common, compatible. Numbers printed in italics denote incompatible combinations. Numbers outside the brackets denote proportions of compatible matings in per cent for the mating categories of Table 3.)

dencies of the two compatibility series, seem to indicate that the incompatibility mechanism in rye may constitute a modification of the Personate type. The following hypothesis has been arrived at to explain the incompatibility relations in rye:

TABLE 3. *Percentage of compatible cross-pollinations in different mating combinations under the assumption of different numbers of Personate incompatibility systems.*

(BC + = female compatible with the parent plant
BC — = » incompatible » » » »)

	A One system	*B* Actual values	*C* Two systems
a. Intercross BC + × S_1	75	82	81,25
b. Backcross S_1 × P	50	77	75
c. Intercross S_1 × BC —	50	74	75
d. » S_1 × S_1	37,5	59	60,9
e. » S_1 × BC +	25	54	56,3
f. » BC — × S_1	0	11	0
g. Backcross P × S_1	0	0	0

P $S_{12}\ Z_{34}$ (selfed)

↓

S_1

♀ \ ♂	$S_{11}\ Z_{33}$ 1	$S_{11}\ Z_{44}$ 1	$S_{22}\ Z_{33}$ 1	$S_{22}\ Z_{44}$ 1	$S_{11}\ Z_{34}$ 2	$S_{22}\ Z_{34}$ 2	$S_{12}\ Z_{33}$ 2	$S_{12}\ Z_{44}$ 2	$S_{12}\ Z_{34}$ 4	
1 $S_{11}\ Z_{33}$	*1*	1	1	1	2	2	2	2	4	
1 $S_{11}\ Z_{44}$	1	*1*	1	1	2	2	2	2	4	
1 $S_{22}\ Z_{33}$	1	1	*1*	1	2	2	2	2	4	
1 $S_{22}\ Z_{44}$	1	1	1	*1*	2	2	2	2	4	81,25 % (*a*)
2 $S_{11}\ Z_{34}$	*2*	*2*	2	2	*4*	4	4	4	8	
2 $S_{22}\ Z_{34}$	2	2	*2*	*2*	4	*4*	4	4	8	
2 $S_{12}\ Z_{33}$	*2*	2	*2*	2	4	4	*4*	4	8	
2 $S_{12}\ Z_{44}$	2	*2*	2	*2*	4	4	4	*4*	8	
4 $S_{12}\ Z_{34}$	*4*	*4*	*4*	*4*	*8*	*8*	*8*	*8*	*16*	0 % (*f*, *g*)

56,3 % (*e*) — 75 % (*b*, *c*)

60,9 % (*d*)

Scheme 2. Incompatibility relations within a parent-offspring group. Two Personate incompatibility factor systems assumed. (Combinations with one or no factor in common, compatible. Numbers printed in italics denote incompatible combinations. Numbers outside the brackets denote proportions of compatible matings in per cent for the mating categories of Table 3.)

TABLE 4. *χ^2-test of frequencies of compatible matings within mating types of Table 3.*

(Letters denote mating categories of Table 3.)

	a		*b*		*c*		*d*		*e*		*f*		*g*	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Expected	39,8	9,2	83,2	27,8	14,2	4,8	77,3	49,7	29,3	22,7	0	19	0	38
Observed	40	9	85	26	14	5	75	52	28	24	2	17	0	38
χ^2	0,0053		0,1388		0,0252		0,1749		0,1321					
P	0,95—0,90		0,80—0,70		0,90—0,80		0,70—0,50		0,80—0,70					

(1) There are two multiallelic incompatibility factor systems cooperating, the S-series and the Z-series (terminology from LAWRENCE, 1930).

(2) The pollen will contain two incompatibility alleles, one from each series. Control of incompatibility is gametophytic. Both alleles must be matched in the style in order to render the pollen incompatible.

(3) The stylar tissue will contain four incompatibility alleles, two from each series. Within the systems these factors are acting independently.

The incompatibility relations, expected under the control of two Personate incompatibility factor systems, which cooperate according to the hypothesis given, are illustrated in Scheme 2, and summarized in Table 3 *C*. As can be seen from Table 4, there is a good agreement between data observed and values expected on hypothesis, it being assumed that the parent plants are heterozygous in both loci, except for mating category *f* (backcross incompatible S_1 plant $\times$ S_1 plants), where the occurrence of two compatible pollinations is against the hypothesis. Both these pollinations were performed on the same mother plant. This individual might have been misclassified as parent incompatible, when tested to its parent plant. In all original parent plants (perhaps with the exception of clone No. 3) data seem to indicate heterozygosity in both loci.

DISCUSSION.

Cooperation of two allelic series of incompatibility factors has been suggested in some previous connections, primarily as an explanation of cases of one way incompatible matings between individuals not derived from selfing. In order to explain SIRKS's (1926) data on *Verbascum phoeniceum*, LAWRENCE (*l. c.*) assumed two series of oppositional alleles — the S-series and the Z-series, respectively — one being more or less a duplicate of the other. A pollen with at least one of its two alleles unmatched in the style was by this hypothesis compatible, but the identity between the two allelic systems was assumed sometimes to be sufficient to permit an incompatibility reaction even between alleles belonging to *different* series.

OWEN (1942) observed one-way sterility in reciprocal crossings in a material of *Beta vulgaris*, and was able to explain the mating results rather satisfactorily after applying to his data the above-mentioned hypothesis on *Verbascum phoeniceum*. Some results also from mating between self-sterile and self-fertile plants followed by double back-

cross to the self-sterile parental plant, could most easily be explained on the assumption of duplicate incompatibility factors.

SEARS (1937), investigating incompatibility in *Brassica oleracea* var. *italica*, was able to confirm the observations of differences in reciprocal crosses (KAKIZAKI, 1930). The latter investigator tried to explain the peculiarities assuming an interaction between two allelic systems, one inhibiting, the other stimulating pollen tube growth. As pointed out by SEARS, there will be a serious obstacle to this interpretation caused by the ability of pollen to grow on unspecialized media. As a substitute, SEARS proposed the existence of two allelic systems, the S-series and the Z-series, respectively, both of the oppositional type with different incompatibility power in their different alleles. One allele of the pollen grain matched in the style, consequently, would sometimes be sufficient to cause incompatibility; both alleles would in other cases have to be matched. Certain allelic identities between the two series would cause further complications.

From *Brassica campestris* also similar occurrence of one-way incompatibility in reciprocal matings is reported (OLSSON, 1953).

From the examples mentioned above the assumption is derived that presence in the pollen of two different incompatibility factors will cause an impairment of the incompatibility reaction. A similar impairment of the incompatibility reaction, has been observed also in the controlled instances of duplicate series of oppositional alleles, offered by artificial autopolyploids of well analysed self-incompatible species. In *Oenothera organensis*, LEWIS (1943) demonstrated that the causes of this impairment are localized to the male gametophyte and not to the stylar tissue. The pollen was formerly adapted to carry only *one* incompatibility factor. *Two* different alleles in the same pollen grain, therefore, will often result in some kind of interaction between the alleles (dominance, competition; LEWIS, 1943, 1947). Depending on genotype and allelic combinations, there will be different degrees of allelic interaction in the pollen, leading to different degrees of incompatibility impairment. At the one extreme of competitive allelic interaction, there will be no incompatibility reaction, even with both alleles of the pollen matched in the style. At the other extreme, the presence of only one allele common to the pollen and the style will still result in a complete incompatibility reaction. LEWIS (1943) suggests that the latter possibility might give the explanation of certain irregularities in the incompatibility data on *Verbascum* and *Beta*. Finally, the reaction of the pollen grain may be completely determined by only one

of its two alleles, which then exhibits some degree of dominance over the other allele.

The existence of two cooperating allelic series of incompatibility factors can obviously be explained in different ways. A new system can arise completely independently and be elaborated in addition to a system already at work. Considering the highly specific character of the incompatibility reaction (LEWIS, 1949), it is not probable, however, that alleles will arise identical with those already existing in the old series. The interpretation of the incompatibility conditions in *Verbascum* and *Beta*, implied at least partial allelic identity between the two series, and in the case of *Brassica* also this possibility is considered (SEARS, *l. c.*). The existence of two series of incompatibility factors is therefore, most probably, the result of a duplication of a more or less radical kind, as far as these species are concerned. *Verbascum phoeniceum* is most probably an allotetraploid species, constituted by two self-incompatible species hybridizing and thereby combining two series of incompatibility factors, and *Brassica oleracea* is at least a partial polyploid (LAWRENCE, *l. c.*). In *Beta vulgaris*, with a somatic chromosome number=18, very little will be found to indicate polyploidy. TISCHLER (1950) assigns 9 to be the basic number of *Chenopodiaceae*, but a few species are found within that family with $n=6$. In haploid *Beta vulgaris* is meiotic pairing non-homologous (LEVAN, 1945). Nevertheless, some kind of duplication within the genome of *Beta vulgaris*, also, seems to be the unavoidable corollary of the incompatibility data given by OWEN (*l. c.*).

Returning to the present case of rye, Table 1 will show no complications which can be traced back to allelic identity between the two series of incompatibility factors. On the whole, the 15 clones have had similar mating reactions, independently of the degree of pseudo-fertility shown in the single instances. The interaction between alleles in the pollen, seems to be regularly competitive. At least, there are no indications of complications, resulting from dominance interaction between the alleles.

By themselves, therefore, the results do not permit definite conclusions as to the origin of the second series of incompatibility factors. They constitute, however, the genetical equivalent to the observation that out of the seven different chromosomes in rye four can be grouped into two pairs (PATHAK, 1940). PATHAK suggests that the basic number of rye was originally 5. Studying the pachytene stage in rye, LIMA-DE-FARIA (1952) demonstrated that corresponding to these similarities

in the somatic chromosomes there are considerable resemblances in the chromomere arrangements of the chromosomes in question. That, however, was not accepted as a definite demonstration of polyploid characteristics in rye, and LIMA-DE-FARIA (*l. c.*) referred to the fact that, probably, meiotic pairing in haploid rye is mainly non-homologous (LEVAN, 1942). The chiasma frequency, however, did indicate that one chiasma was more easily formed than the rest of the chiasmata, and that fact might be taken as an indication of the presence of one or two large duplications within the genome of rye (LEVAN, *l. c.*).

The problem of the origin of the second series of incompatibility factors in rye, can not be solved on the present material, but at least, it can not be excluded that here, too, it might be connected with some kind of duplication. Anyhow, it might be questioned how it is possible for two series of incompatibility factors to exist side by side, obviously in a very regular cooperation: loss mutations and differentiation in a new direction would tend to eliminate the incompatibility controlling action in one of the series, provided the biserial system is lacking special selective advantage.

As an integral part of the breeding system of a flowering plant species an incompatibility system will primarily prevent self-fertilization and restrict the fusion of nuclei from individuals closely related to one another or else of a similar genotype. Outbreeding is encouraged. The immediate consequence will be a restriction of the frequency of inferior genotypes, which result from random interbreeding through the mating of genotypes that are similar owing to close relationship or mere chance. The incompatibility, however, might also affect combinations where neither relationship, nor genotype, in themselves warrant it. Interbreeding is no longer free, certain mating combinations are favoured, and so the incompatibility system might to a certain degree counteract itself. This restriction of randomness of interbreeding is inverted to the number of different incompatibility alleles of the population, and, consequently, it is of great importance for the population to contain several different incompatibility alleles (*cf.* MATHER, 1944).

The average frequency of individual incompatibility alleles in the population, will, however, decrease with an increasing number of different alleles, and random drift due to the accidents of sampling, will lead to an increased elimination of alleles from the population (WRIGHT, 1939). It will obviously be an advantage if an incompatibility system can combine the relatively unimpaired random interbreeding following a polyallelic system, and the stability against random drift, associated

with an oligoallelic system. Under certain assumptions these advantages can be associated with an incompatibility system, where the factors are apportioned to two allelic series.

The apportionment of a given number of incompatibility alleles to one and two factor series, respectively, is assumed. In the case of two incompatibility factors in the pollen, the assumption is retained from the hypothesis proposed above, that both of them must be matched in the style in order to produce a complete incompatibility reaction. Under the simplifying assumption that, within each single series, the alleles given are equally frequent, and that their corresponding possible zygotic combinations in the population are equally frequent, too, the number of incompatibility alleles in the population at one series will be represented by n; and at two series by n_1 and n_2, respectively. The following comparison will show frequencies of different kinds, calculated on these assumptions.

Number of possible	At one series	At two series
a. Pollen genotypes	n	$n_1 n_2$
b. Style genotypes	$\frac{n(n-1)}{2}$	$\frac{n_1 n_2 (n_1 + n_2 + n_1 n_2 - 3)}{4}$
c. Incompatible combinations between pollen and style	$n(n-1)$	$n_1 n_2 (n_1 n_2 - 1)$
d. Total combinations between pollen and style	$\frac{n^2(n-1)}{2}$	$\frac{n_1^2 n_2^2 (n_1 + n_2 + n_1 n_2 - 3)}{4}$

The ratio c/d will express the restriction of random interbreeding. The difference between the two incompatibility systems will be rather small at a high number of incompatibility alleles, will have its maximum at a moderate number (8—20), and will again decrease at a still lower number of factors. With an unequal apportionment of alleles to the two series of incompatibility factors, the difference between the monoserial and the biserial systems will be reduced. In the extreme case, one of the two series being represented by a single allele, the function of the biserial system is, in fact, that of a monoserial system reduced by one allele.

The differences between the monoserial and the biserial systems will, on the whole, be rather insignificant, however: of the total number of possible interbreeding combinations, 5—10 per cent more will be incompatible at the monoserial system, maximally. On the other hand,

the stability against sampling accidents, shown by the biserial system, seems to be more important. The very fact that, by hypothesis, every individual will contain at least three different incompatibility alleles, will mean a better guarantee against losses of alleles, caused by random drift. Moreover, the single alleles will here be represented in proportionally more individuals than will be possible with a monoserial system. Following the assumptions above, a certain allele will be represented in the population according to the following expressions:

$$e.\ \frac{2(n-1)}{n(n-1)} = \frac{2}{n} \quad = \text{proportions at one series of factors}$$

$$\left.\begin{array}{l} f.\ \dfrac{2(n_1 + n_1n_2 - 2)}{n_1(n_1 + n_2 + n_1n_2 - 3)} \\ g.\ \dfrac{2(n_2 + n_1n_2 - 2)}{n_2(n_1 + n_2 + n_1n_2 - 3)} \end{array}\right\} = \text{proportions at two series of factors}$$

Especially at low and moderately high numbers (up to about 20) of incompatibility alleles, the average occurrence of alleles in the population will be considerably higher at a biserial incompatibility system than at a monoserial one. Differences between the systems amounting to 10—30 per cent of the total number of individuals in the population can be obtained now. Unequal apportionment to the two series of the total number of the incompatibility factors of the population, will of course lead to the individual alleles in the larger series becoming less common, whereas the alleles of the smaller series will be correspondingly better represented in the population. On an average, however, the biserial system will be strongly superior to the monoserial system.

It may now be of interest to draw attention to the selective advantage, that competitive allelic interaction in the pollen may have in autopolyploids in relation to the dominance interaction (BREWBAKER, 1953). Autopolyploid populations are often derived from a restricted parental material, resulting in a more frequent inbreeding, and dominance pollen will then not be able to function as often as competition pollen. Self-fertile combinations are selectively favoured (BREWBAKER, *l. c.*). A prerequisite for this selective advantage of self-fertility, is the increased resistance to inbreeding, shown by polyploids (*e. g.*, LUNDQVIST, 1947, 1953). Rye being a diploid organism, selection has here followed a different path. Self-fertility will now be disadvantageous owing to the bad effects of inbreeding, and therefore selection seems to have favoured a rather moderate allelic competition in the pollen. The incompatibility reaction is impaired, certainly, and one allele of the

pollen matched in the style will not be sufficient to cause inhibition of pollen tube growth; but two impaired incompatibility reactions added to each other, when both alleles of the pollen are matched in the style, will be sufficient for a complete incompatibility reaction. Self-fertilization is still prevented, and at the same time the population will have its supply of incompatibility alleles more safely guarded and thus ensure an effective interbreeding.

Acknowledgement. — The author is much indebted to Dr. G. ÖSTERGREN for directing his attention to the possibility of a selective advantage of a biserial incompatibility system.

SUMMARY.

(1) An analysis of self-incompatibility was tried with 15 clones of »Steel-rye». S_1 plants were reciprocally backcrossed to their parent plants, and intercrossed.

(2) Different gradations of self-incompatibility seemed to be strongly genetically conditioned.

(3) The frequency of compatible mating combinations was considerably higher than is to be expected on other incompatibility systems, so far recognized. Affinities to the Personate system were revealed.

(4) A hypothesis is proposed, that incompatibility in rye is controlled by two allelic series of incompatibility factors, both acting on the Personate scheme. Competitive interaction between factors in the pollen is made probable.

(5) The origin of the new series of incompatibility factors through a duplication is not excluded.

(6) It is suggested that, under certain conditions, the biserial incompatibility system may have a selective advantage as compared to a monoserial system.

LITERATURE CITED.

1. BREWBAKER, H. E. 1926. Studies of self-fertilization in rye. — Tech. Bull. Minn. Agric. Expt. Sta. 40; 40 pp.
2. BREWBAKER, J. L. 1953. Oppositional allelism in diploid and autotetraploid *Trifolium hybridum* L. — Genetics 38: 444—455.
3. CROWE, L. K. 1954. Incompatibility in *Cosmos bipinnatus*. — Heredity 8: 1—11.

4. EAST, E. M. and MANGELSDORF, A. J. 1925. A new interpretation of the hereditary behavior of self-sterile plants. — Proc. Nat. Acad. Sci. Wash. 11: 166—171.
5. GERSTEL, D. U. 1950. Self-incompatibility studies in guayule. II. Inheritance. — Genetics 35: 482—506.
6. HUGHES, M. B. and BABCOCK, E. B. 1950. Self-incompatibility in *Crepis foetida* (L.) subsp. *rhoeadifolia* (BIEB.) SCHINZ et KELLER. — Genetics 35: 570—584.
7. KAKIZAKI, Y. 1930. Studies on the genetics and physiology of self- and cross-incompatibility in the common cabbage (*Brassica oleracea* L. var. *capitata* L.). — Jap. Journ. Bot. 5: 133—208.
8. LAWRENCE, W. J. C. 1930. Incompatibility in polyploids. — Genetica XII: 269—296.
9. LEVAN, A. 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII: 177—211.
10. — 1945. A haploid sugar beet after colchicine treatment. — Hereditas XXXI: 399—410.
11. LEWIS, D. 1943. Physiology of incompatibility in plants. III. Autopolyploids. — Journ. of Genetics 45: 171—185.
12. — 1947. Competition and dominance of incompatibility alleles in diploid pollen. — Heredity 1: 85—108.
13. — 1949. Incompatibility in flowering plants. — Biological Reviews 24: 472—496.
14. LIMA-DE-FARIA, A. 1952. Chromomere analysis of the chromosome complement of rye. — Chromosoma 5: 1—68.
15. LJUNG, E. W. 1921. Svalöfs Stålråg. — Sveriges Utsädesförenings Tidskrift XXXI: 95—101.
16. LUNDQVIST, A. 1947. On self-sterility and inbreeding effect in tetraploid rye. — Hereditas XXXIII: 570—571.
17. — 1953. Inbreeding in autotetraploid rye. — Hereditas XXXIX (Festskrift för ARNE MÜNTZING 2.3.1953): 19—32.
18. MATHER, K. 1944. Genetical control of incompatibility in Angiosperms and Fungi. — Nature 153: 392—394.
19. MÜNTZING, A. 1943. Double crosses of inbred rye. — Botaniska Notiser 1943: 333—345.
20. NILSSON, HERIBERT. 1916. Populationsanalysen und Erblichkeitsversuche über die Selbststerilität, Selbstfertilität und Sterilität bei dem Roggen. — Ztschr. f. Pflanzenzüchtung IV: 1—44.
21. — 1953. Über die Entstehung der Selbstfertilität beim Roggen. — Hereditas XXXIX (Festskrift för ARNE MÜNTZING 2.3.1953): 65—74.
22. OLSSON, G. 1953. Undersökning av självfertiliteten hos artificiell raps. — Kungl. Lantbruksakademiens Tidskrift 92: 394—402.
23. OWEN, F. V. 1942. Inheritance of cross- and self-sterility and self-fertility in *Beta vulgaris*. — Journ. of Agric. Res. 64: 679—698.
24. PATHAK, G. N. 1940. Studies in the cytology of cereals. — Journ. of Genetics 39: 437—467.
25. PETERSON, R. F. 1934. Improvement of rye through inbreeding. — Scientific Agriculture XIV: 651—668.
26. SEARS, E. R. 1937. Cytological phenomena connected with self-sterility in the flowering plants. — Genetics 22: 130—181.

27. SIRKS, M. J. 1926. Further data on the self- and cross-incompatibility of *Verbascum phoeniceum*. — Genetica 8: 345—367.
28. TISCHLER, G. 1950. Die Chromosomenzahlen der Gefässpflanzen Mitteleuropas. — s'-Gravenhage; 263 pp.
29. ULRICH, K. 1902. Die Bestäubung und Befruchtung des Roggens. — Inaugural-Diss. (Jena) Halle/a. S., pp. 1—62.
30. WRIGHT, S. 1939. The distribution of self-sterility alleles in populations. — Genetics 24: 538—552.

CONTENTS.

Material and methods 278
Experimental results 280
Selfings 280
Cross-pollinations 281
Genetic interpretation 282
Discussion 286
Acknowledgement 292
Summary 292
Literature cited 292

EFFECT OF OXYGEN ON IRRADIATED MALES AND FEMALES OF DROSOPHILA

BY *K. G. LÜNING*
INSTITUTE OF GENETICS, UNIVERSITY OF STOCKHOLM

I. INTRODUCTION.

WITHIN the last few years much interest has been concentrated on the variations in differential sensitivities to ionizing irradiation which have been found in both plants and animals. These variations may be inherent in that they depend on processes or physiological states in the test object which, while sometimes recognized, can not always be controlled, or they may be induced by different chemical and physical agents. The variations may be in reverse directions: increasing or decreasing the effects of the irradiation.

Particular interest has been paid to the protection action of low oxygen concentrations during the period of irradiation. Two hypotheses have been proposed to explain this protective action: (a) At low oxygen concentrations there are induced fewer primary chromosome breaks than in higher oxygen concentrations. (b) There is induced the same rate of primary breaks but low oxygen concentrations favour the rejoining of broken ends. The results of earlier investigations have been interpreted as favouring the first hypothesis (THODAY and READ, 1949; GILES, 1952). Later experiments by SCHWARTZ (1952) with maize have been interpreted in terms of the latter hypothesis. In experiments with *Drosophila*, BAKER and VON HALLE (1953) have also considered their results as supporting the latter hypothesis.

There is, however, a fundamental difference between the original statement of SCHWARTZ and the later statements of BAKER and VON HALLE although both support the »rejoining hypothesis». SCHWARTZ has stated: »Chromosomes broken in the absence of oxygen are more capable of rejoining than chromosomes broken in the presence of oxygen». This means that the rate of breaks which remain unjoined is lowered in absence of oxygen. BAKER and VON HALLE, however, state that »... the breaks induced in N_2 are more likely to rejoin and to rejoin more quickly than those induced in air. Since the only type of rejoining possible in the sperm is restitution (excluding sister-strand fusion if the chromosome is split) the end result would be fewer breaks available at the time

of fertilization to form new arrangements», and further that »... although the O_2 concentration affects the ability of a broken chromosome end to restitute in the sperm, it does not influence its ability to rejoin at the time of fertilization». This means that the rate of breaks which remain unjoined, per break available at fertilization, is *not* affected by oxygen concentration. From these citations it is clear that they discuss different processes in a similar vocabulary, with a consequent confusion of the concepts. In order to arrive at an understanding of the oxygen action it is necessary to distinguish between an effect on the general rejoinability of broken ends (rejoining of broken ends in the original or in a new order; SCHWARTZ) and on a time-limited restitution (rejoining only in the original order) having the general rejoinability at the time of fertilization unchanged (BAKER and VON HALLE).

As the discussion by BAKER and VON HALLE is based on the opinion that there is no differential sensitivity in sperm, their results are open to a reinterpretation since it has been demonstrated that there is a differential sensitivity among the chromosomes in the sperm of *Drosophila,* a sensitivity which depends both on the age of the males at treatment and on the time between treatment and insemination (LÜNING, 1952, a and b).

The present paper deals with an analysis of the oxygen action on X-ray induced chromosome breaks in *Drosophila* sperm studied by one type of mutation known to be due in the main to minute rearrangements and another type of aberration due to a gross deficiency. There are also included some studies on the oxygen action on irradiated females.

II. MATERIAL AND METHODS.

In the studies of the effect of different oxygen concentrations on induced dominant lethals and vital rearrangements in *Drosophila* sperm, Muller-5 (*M5*, sc^{S1} *B In S* w^a sc^8) males were irradiated. In tests of dominant lethality it is desirable to use females which lay large numbers of eggs having a high rate of hatchability when mated to non-irradiated males. According to STRÖMNÆS (1949), F_1 daughters from *cn bw*; e^{11} ♀♀×Canton S ♂♂ fulfil this requirement. Dr. STRÖMNÆS has kindly placed these stocks at my disposal. This type of F_1 daughters, referred to as »egglaying» females, has been used both as mates to irradiated males, and in the second part of the investigation, they have themselves been irradiated and mated to non-irradiated males of the Canton S stock.

In order to study vital rearrangements induced in spermatozoa, M5 males have been mated to *y w sn* females, and the F_1 offspring have been analysed.

As I intended to study vital rearrangements induced in eggs, I would use the same types as in the experiments with irradiated spermatozoa. Consequently, I ought to irradiate M5 females and mate them to *y w sn* males of the same stocks as mentioned above.

This use of M5 females is, however, hazardous since there is not only a high rate of induced yellow mutations but there is also a high rate of spontaneous yellow mutations in Muller-5 chromosomes. As most of these yellow mutations are recessive lethals, they would not be detected in the *M5* stock but would appear when *M5* females are mated to yellow males, thus giving clusters of yellow individuals independent of irradiation. One can avoid this drawback by a special technique which makes use of the fact that the lethality is mostly balanced by the $sc^8\,Y$ chromosome which contains $y^+\,ac^+$ loci from a sc^8-*X* chromosome. If the y^+ allele in the $sc^8\,Y$ is mutated to *y*, males containing a lethal yellow mutation in the *X* chromosome and having the $y\,sc^8$-*Y* chromosome would be vital and phenotypically yellow. By this technique it is possible to detect most yellow mutations besides those few which are lethal also with $y\,sc^8\,Y$. During the experiment 2 yellow males were detected in the stock. No clusters of yellow mutations were found in the experiments, indicating that there were no females heterozygous for a spontaneous yellow mutation. Virgin females are taken from this $M5 \times M5$; $y\,sc^8\,Y$ stock. After irradiation they were mated to *y w sn* males.

The flies were irradiated in a wooden box with a plexiglass top and through which was passed a stream of air or of commercial nitrogen. In the latter case they were left 10 minutes in nitrogen before irradiation with x-rays from a Skandia Intensiv apparatus operated at 170 kv, 15 m A, and with an inherent filtration equivalent to 2 mm Al. The dose was 3240 r given in 6 min. Stocks and cultures were reared on yeast suspension as described by the author (LÜNING, 1952 b). They were incubated at $25° \pm 1°$ C.

III. EXPERIMENTS.

1. MALE IRRADIATION SERIES.

The most striking results in the investigation by BAKER and VON HALLE (1953) were that when irradiation of males was carried out in an atmosphere of nitrogen the same rates of dominant lethals were

TABLE 1. *The effect of oxygen concentration on the rate of dominant lethals induced in spermatozoa inseminated the first or the second day after irradiation of 0—1 day old Muller-5 males mated to F_1 daughters from the cross cn bw; e^{11} ♀♀ × Canton S ♂♂. Eggs were collected the day after removal of the males.*

Atmosphere	Period of insemination in days after treatment	Total number of eggs	% hatched
Air	1	905	45,6
»	2	664	58,1
Nitrogen	1	891	57,6
»	2	854	60,3

induced in sperm inseminated in the first 24 hours after irradiation as in the following 24—48 hour interval, while when irradiation in air induced more dominant lethals in the first sperm batch than in the second. — A similar difference between the 1st and 2nd day sperm batch irradiated in air had been observed in this laboratory (LÜNING, 1952, p. 98), but these studies were more concerned with variations at another time interval after irradiation, and the significance of the difference in the first two sperm batches was not further studied at that time.

The first task was to verify the results of BAKER and VON HALLE in a rather small experiment using the same criterion of effect as they had used, *viz.* dominant lethals.

For reasons which will be given below 0—1 day old Muller-5 males were irradiated either in air or in an atmosphere of nitrogen. The males were immediately mated to »egglaying» females in pairs, prior etherization the males were transferred to new virgin females 24 hours later. They were discarded after another 24 hours. The females were allowed to lay eggs the day following removal of the males, and only cultures with larvae were counted. The results are presented in Table 1. As can be seen, these data agree with the results of BAKER and VON HALLE.

Dominant lethals are generally presumed to be due to two different chromosomal causes (LEA and CATCHESIDE, 1945), *i. e.*, breaks which remain unjoined and breaks which rejoin in asymmetrical interchanges. Changes in the rate of dominant lethals could be due to one or both of these two causes. It has been shown (LÜNING, 1952 b) that

variation in the rates of induced dominant lethals as a function of the time between irradiation and insemination was paralleled by a variation in the rates of gross rearrangements (hyperploid males), indicating that the variation in the rates of dominant lethals was due to both causes mentioned above.

By using the same technique, in order to take advantage of the occurrence of hyperploid males, it is possible to study the cause of the different rates of dominant lethals in spermatozoa inseminated the 1st and the 2nd day after irradiation in air in contrast to the lack of difference between the two batches resulting from irradiated in nitrogen. BELGOVSKY (1938) showed that, in chromosomes with large inversions, with one break close to yellow locus and the other in the proximal heterochromatic region, there are induced higher rates of yellow mutations than in wild type chromosomes. Most of these yellow mutations are also achaete and behaved as recessive lethals. This type of yellow mutations was found to be due mainly to minute rearrangements (see review by LÜNING, 1952 c). By using Muller-5 males, it is therefore possible to study both minute and gross rearrangements in the same experiment.

The material to be collected for the particular problems mentioned above could also be used to elucidate quite another problem related to the earlier studies of variation in the breakability of chromosomes in sperm inseminated up to 10 days after irradiation. The cause of this natural variation could be due possibly to differences in the oxygen concentration in various stages of spermiogenesis. To investigate this aspect of variation, use could be made of the results from spermatozoa inseminated within the first days after irradiation in nitrogen, and to these could be added a series where the same males were mated also at the 7th to the 10th day after irradiation. As the maximum difference between the rates of chromosome breaks induced in spermatozoa inseminated the first days as compared to the 7th to 10th day appeared when 0—1 day old males were irradiated in air (LÜNING, 1952 b), it was convenient to irradiate the Muller-5 males in the present experiment when 0—1 day old. Immediately after irradiation the males were mated to *y w sn* females (6—8 pairs per vial). 24 hours later the males were transferred to a new group of virgin females for 2 days. Presumably the second mating should have been for a 24- instead of a 48-hour period, but as the rate of dominant lethals induced in spermatozoa inseminated during the 2nd and 3rd day is about the same (LÜNING, 1952 a) it was technically easier to conduct the experiment in this way.

TABLE 2. *Offspring from crosses y w sn ♀♀ × M5 ♂♂. The males were irradiated in air or in nitrogen when 0—1 day old and immediately mated. 24 hours later they were transferred to new virgin females for another 2 days and in the nitrogen series they were mated a third time from the 7th to the 10th day after irradiation.*

Atmosphere	Period of insemination in days after treatment	Total offspring from X-carrying spermatozoa	Yellow		Fractional yellow		Total yellow		Hyperploid males	
			Abs. n:r	per 10^4	Abs. n:r	per 10^4	Abs. n:r	per 10^4	Abs. n:r	per 10^4
Air	1	25194	56	22,2	13	5,2	69	27,4	23	9,1
»	2—3	29060	43	14,8	5	1,7	48	16,5	15	5,2
Nitrogen	1	42708	70	16,4	12	2,8	82	19,2	22	5,2
»	2—3	46021	45	9,8	9	2,0	54	11,7	13	2,8
»	7—10	14465	41	28,3	1	0,7	42	29,0	15	10,4

In the »nitrogen» series the males were allowed to mate a third time between the 7th and 10th day. Eggs were collected during the period of mating and also in some cases 3 days after the removal of the males. The F_1 daughters were examined, and full and fractional yellow mutants were recorded as well as hyperploid males, the latter appearing phenotypically grey instead of yellow. The results are presented in Table 2. It is immediately evident that in the series irradiated both in air and in nitrogen there are more aberrations induced in spermatozoa inseminated the 1st day as compared to those from the 2nd and 3rd day, except for fractional yellow mutations in the nitrogen series. It is believed that this exception is of no significance because of the low absolute number of this type of aberration. As both yellow mutations and hyperploid males are supposedly due to breaks, it is permissible to combine the aberrations and compare the total rate of aberrations induced in the various series. Comparing »Air 1» to »Air 2—3», one gets by the angular transformation method $0{,}01 > P > 0{,}001$ which is in agreement with CATSCH and RADU (1943) and »Nitrogen 1» to »Nitrogen 2—3», $P < 0{,}001$, »Air 1» to »Nitrogen 1», $0{,}01 > P > 0{,}001$ and »Air 2—3» to »Nitrogen 2—3», $0{,}03 > P > 0{,}02$.

There is therefore a disagreement between the results which BAKER and VON HALLE (1953) have found with dominant lethals, which have also been confirmed (Table 1), and the data on vital rearrangements (Table 2) obtained after irradiation in an atmosphere of nitrogen. The significance of this will be discussed below.

The data in Table 2 also permit a consideration of whether the normal variations in breakability of chromosomes in various stages of spermiogenesis irradiated in air are paralleled by similar variations when irradiation is carried out in nitrogen. It can be seen that in sperm inseminated 7—10 days after irradiation there are induced more full yellow mutations and hyperploid males, but fewer fractional yellows, as compared with sperm inseminated the 1st or the 2nd to the 3rd day. The discrepancy between the rates of full and fractional yellow mutations in this comparison may seem strange. Similar observations, however, have been made on another type of mosaicism, namely gynandromorphs (LÜNING, 1952 b), which did not vary as other break-dependent aberrations — *i. e.*, dominant lethals and hyperploid males — when induced in various stages of spermiogenesis. At that time the results were rather confusing since gynandromorphs were supposed to be due to breaks which had remained unjoined in one chromatid followed by the subsequent loss of this portion of the chromatid (BONNIER, LÜNING and PERJE, 1949). In order to explain this it was stated: »This indicates that there is — at least in some stages — induced breaks that are not associated with the occurrence of gynandromorphs and gene mutations» (LÜNING, 1952, p. 335). It is believed that a reconsideration of this problem is now possible by comparing gynandromorphs with fractional yellow mutants.

In the present experiment several of the fractional yellow mutants have been tested. In some cases the gonads have contained the mutant chromosome — which in most cases has also been *ac* and behaved as recessive lethals. In others the gonads contained a non-yellow M-5-chromosome, which was neither *ac* nor recessive lethal. A few cases have been gonad mosaics. The *y* mutants have in most cases also been *ac* and recessive lethals while all non-*y* chromosomes were vital.

In a great number of tested full yellow mutants it was found that most of them were also *ac* and recessive lethals, as SIDOROW (1936) showed. It made no difference whether they were produced by irradiation in air or in nitrogen.

It may be suggested, therefore, that yellow mutations which appeared as fractionals as well as those which appeared as full mutants originated by similar processes, but that in fractionals the mutation had been confined to one chromatid only. The failure of fractional yellow mutations in the series »Nitrogen 7—10» can be explained if it is supposed that the fate of breaks induced at this stage — supposed to be spermatids (LÜNING, 1952 a) — tend to be the same for both chro-

matids at fertilization. This explanation would also be true for the failure to realize the expected increase in the rate of gynandromorphs mentioned above, and can consequently replace the hypothesis proposed previously to account for this failure (LÜNING, 1952 b).

The results in Table 2 can now be examined in so far as they concern the variation in the rates of full yellow mutants and hyperploid males in the three »nitrogen» series. Series »Nitrogen 7—10» is significantly different from both »Nitrogen 1» and »Nitrogen 2—3». It means that there are induced more breaks in sperm inseminated 7—10 days than in the first 3 days. This is in agreement with my results (LÜNING, 1952 b) after irradition in air.

As these variations in breakability appear after irradiation both in air and in nitrogen the cause of their appearance can not be due to differences in oxygen concentration at the various stages of spermiogenesis. It is, however, possible that the amplitude of the variation can be influenced by the oxygen concentration but the present material is insufficient to determine whether any significant difference exists.

2. FEMALE IRRADIATION SERIES.

SONNENBLICK (1940) reported that when females were irradiated eggs laid within 24 hours after treatment showed a higher mortality than was found when sperm were exposed to the same dose. KING (1952) showed that the productivity, taken as an inverse measure of dominant lethals, was lowest in the 1st day and then increased gradually, although not continuously, until the control value was approached 7—12 days after irradiation. WHITING (1940) reports in *Habrobracon* a higher sensitivity in prometaphase than in earlier stages. These data are in agreement with the results of SONNENBLICK and KING on *Drosophila.*

It was of interest, therefore, to confirm KING's inverse measure of dominant lethals by a direct study of this type of aberration, and to place the results in proper relation to those presented in the first part of this investigation. This was done by irradiating females both in air and in nitrogen. Since there are uncertainties as concerns the fertilization of the first eggs laid this study was confined to an investigation of eggs laid 2—4 days after irradiation. 2—4 days old »egglaying» females (see above) were treated and immediately mated singly to Canton S males in vials which were numbered. Eggs were collected 4 consecutive days from each female. Eggs laid the 1st day were not

TABLE 3. *F_1 daughters from the cross cn bw; e^{11} ♀♀×Canton S ♂♂ were irradiated and immediately mated to Canton S ♂♂. Eggs were collected 4 consecutive days. The hatchability was examined only if there had appeared larvae from eggs laid the preceding day.*

Atmosphere	Days after treatment					
	2		3		4	
	Total eggs	% hatched	Total eggs	% hatched	Total eggs	% hatched
Air	1303	64,7	2168	66,7	1935	74,5
Nitrogen	1260	88,5	2239	89,8	2226	93,4

Control: Out of 4737 eggs laid, 96,16 % hatched.

counted, but the occurrence of larvae was recorded. Eggs laid the 2nd, 3rd and 4th day were counted if there had appeared larvae in a preceding day. There was also one control series. The results are presented in Table 3.

The results were remarkable in that the rate of dominant lethals was lower (higher hatchability) than in the corresponding experiment with males given the same dose, Table 1. Part of the discrepancy can be accounted for by the use of M5 males in the male irradiation series, but without doubt there is a real difference. Even more remarkable was the strong protective effect of nitrogen as compared to its rather low protection effect in males.

Before discussing these data, however, it is desirable to examine the results of studies of vital rearrangements, yellow mutations and hyperploid males in Muller-5 females from the *M5*×*M5*; *y* sc^8 *Y* stock. These females were irradiated without etherization at an age of 2—6 days and were, again without etherizaton, put into vials (8—12 per vial) together with *y w sn* males. The females were allowed to lay eggs 6 consecutive days divided in 3 equal periods: *i. e.*, 1—2, 3—4 and 5—6 days after irradiation. The results are presented in Table 4.

There appeared only *one* hyperploid in the entire experiment. This male appeared among the first flies hatching from eggs laid 1—2 days after irradiation and must, as a consequence, have arisen from a mutation induced in a mature egg.

There appeared only a few fractional yellow mutants. They are included in the full yellow group.

The results are not as expected since there is induced a high rate of yellow mutants in the series treated in air as compared to the corres-

TABLE 4. *Offspring from crosses M5* ♀♀ × *y w sn* ♂♂. *The females were irradiated when 2—6 days old either in air or in nitrogen and were immediately mated. Eggs were collected in 2 days periods during 6 days.*

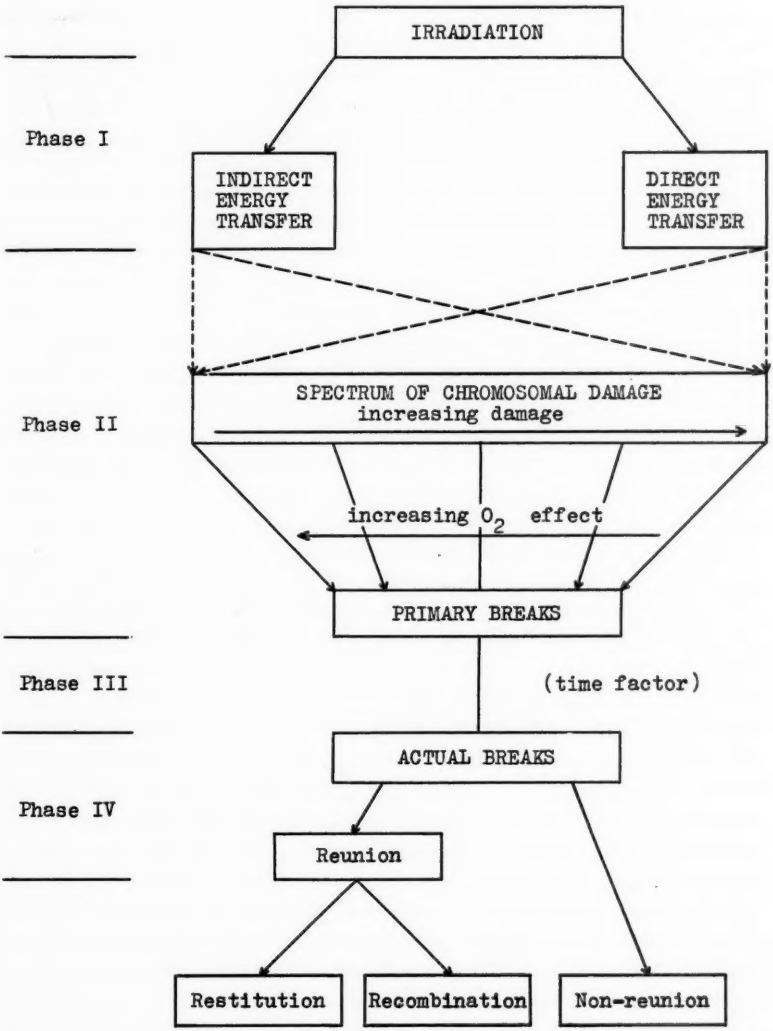
Atmosphere	Period of egglaying in days after treatment	Total daughters	Yellow		Hyperploid males	
			Abs. n:r	per 10^4	Abs. n:r	per 10^4
Air	1—2	13259	52	39,2	1	0,8
»	3—4	11924	40	33,5		
»	5—6	17566	43	24,5		
Nitrogen	1—2	9268	10	10,8		
»	3—4	20017	23	11,5		
»	5—6	15999	8	5,0		

ponding rates of dominant lethals. The strong protective action of nitrogen was evident also as concerns vital rearrangements. These results will be discussed below.

IV. DISCUSSION.

Two different hypotheses have been proposed to explain the protective action of anoxia during irradiation, *viz.* the breakage and the reunion hypotheses. In the introduction, where these hypotheses were briefly reviewed, it was stated that in the »reunion» hypothesis one had to distinguish between an effect on restitution (restorating the original order) occurring before fertilization and on rejoinability of broken ends (in original or new orders) at fertilization. To facilitate an understanding of this difference one can use LEA and CATCHESIDE's nomenclature: α, the average number of breaks induced (sperm) 1000 r, which are available for joining at the time of fertilization; q the probability that a break will rejoin at fertilization (either in original or new order).

If now the effect of anoxia is on restitution prior to the period of recombination this would mean a decrease in α. The same result would be achieved if anoxia affected the rate of primary breaks induced (the breakage hypothesis). The proportion of breaks available at fertilization, which remain unjoined or which undergo sister chromatid union, will not be altered by a change in α. If the effect of anoxia is instead on rejoinability at the time of fertilization — *i. e.*, on q — the proportion of unjoined breaks will be changed. An increase in q would lead



Scheme 1. A schematic diagram showing the possible effects of oxygen tension in radiation induced chromosomal aberrations. (For further explanation, see text.)

to a subsequent decrease in the rates of aberrations due to unjoined breaks and an increase in the rates of recombinations, assuming that the proportion of broken ends joining in the original or in new order is not changed. On the other hand, q can decrease and consequently the rates of aberrations due to unjoined breaks increase and the rates of recombinations decrease. With changes of q in either direction, we have, therefore, reverse changes in the two classes of aberrations which are dependent on unjoined and rejoined broken ends. This point has not been discussed by SCHWARTZ (1952) in his consideration of the origin of the differential decreases he obtained in the two types of aberrations studied.

These considerations make it clear that the »breakage» and »reunion» hypotheses are ambiguous, since it is shown that a change in α can occur in two ways (actually three, as shall be pointed out later), and that a change in q only reflects a change in rejoinability at the time of recombination and excludes earlier recovery processes. In order to get a clearer picture of the levels at which anoxia can exert its effect, these are presented diagrammatically in Scheme 1; it has been worked out in cooperation with Dr. C. P. SWANSON. We have compared results of radiation experiments from *Tradescantia* and *Drosophila* from a theoretical point of view in order to construct a diagram which, with minor variations, may be adapted to various experimental organisms.

The results of STONE, WYSS and HAAS (1947) on the irradition of broth prior to incubation indicated that there are produced mutagenic substances which can, at a later time, affect the mutation rates of bacteria. This is consequently an indirect effect of irradiation. Besides this we cannot exclude a direct energy transfer by irradiation which would, by definition, be independent of oxygen concentration, while indirect energy transfer could possibly be affected by anoxia. (Phase I.)

Between the taking up of energy by the chromosome molecule and the realization of an actual break there might occur different processes which could be influenced in different degrees by anoxia, leading to repair. From the results of *Tradescantia*, SWANSON (in press) proposes different degrees of damages based on the results with radiations of different ion densities. (Phase II.)

If there is a time interval between production of primary breaks and their utilization in recombinations — as in *Drosophila* — anoxia might influence the process of recovery—restitution, leading to a decrease in the number of actual breaks available for recombination. (Phase III.)

The fourth possibility is an effect on the rejoinability of the ends of actual breaks. (Phase IV.)

A change in α can occur if the effect of anoxia is in any of the phases I, II and III. The possibility of distinguishing between an effect in I and II *versus* III will be discussed below. A change in q indicates that anoxia acts in phase IV.

By comparing the proportion of breaks which remain unjoined after irradiation in presence of oxygen and in anoxia it would be possible to decide if α or q is changed. In *Drosophila* there is no class of aberrations which consists solely of unjoined breaks or of sister chromatid union of broken ends. They form, however, the major part of dominant lethals, which moreover also includes asymmetrical interchanges, *e. g.* dicentrics and internal deficiencies. Since it may be presumed that lethal interchanges and vital interchanges occur by the same processes one can get a measure of the proportion of dominant lethals due to asymmetrical interchanges by studying the rates of vital rearrangements in relation to the rates of dominant lethals (LÜNING, 1952 b).

BAKER and VON HALLE (1953) showed that after irradiation in air there were more dominant lethals induced in the first sperm batch as compared to the second. This was in agreement with my results (LÜNING, 1952 a) and is also confirmed by the data in Table 1. The rates of minute and gross rearrangements induced in the corresponding sperm batches are included in Table 2.

Parallel with the decrease in the rates of dominant lethals there is a decrease in the rates of vital rearrangements. This is in agreement with other variations in a later period after insemination (LÜNING, 1952 b). This decrease also indicates that this variation in the rates of aberrations is due to a change of α rather than in q.

In the case of irradiation in nitrogen BAKER and VON HALLE obtained the same rates of dominant lethals in the first and the second batch, with both somewhat lower than the rates in the second batch treated in air. This likewise is confirmed by the data in Table 1. The corresponding rates of vital rearrangements in the first and the second batch (Table 2) are significantly different. To get informations about the values of α and q we have to compare the results of irradiation in air and in nitrogen in each sperm batch, since there is a difference between the two batches after treatment in air.

In the first batch the rates of dominant lethals and vital rearrangements are reduced to about the same degree, indicating that

there is a change in α rather than in q. In the second batch the rates of dominant lethals are reduced to a much less degree than are the rates of vital rearrangements. This means that the proportion of dominant lethals due to asymmetrical interchanges is lower after treatment in nitrogen than in air, and consequently the proportion of breaks which remain unjoined is higher in the nitrogen series than in the air series. If the decrease in the rates of vital rearrangements had been due solely to a change in q, with α, remaining unchanged, the rate of dominant lethals would in fact have been higher in the nitrogen series than in the air series, which is not the case. For that reason we must also suggest a decrease in α as was suggested in the first sperm batch. More data would be needed to determine whether the proportional decrease in α is the same in both batches.

Summing up the results, it seems reasonably certain that the effect of anoxia on the production of aberrations in *Drosophila* sperm is in phases I, II or III and in one case (the second sperm batch) *also* in phase IV. It was pointed out above that the results of SCHWARTZ (1952) on maize are not consistent with the hypothesis that the effect of anoxia is solely on the rejoinability of broken ends—phase IV. If there is a change in both the number of breaks (α) and the rejoinability of broken ends (q) the results are explicable. Hence, in maize there could be an effect of anoxia in any one of phases I, II or III as well as in phase IV, as in the second sperm batch in *Drosophila*, but in maize q was increased while in *Drosophila* q was decreased in value.

BAKER and VON HALLE interpreted their results as indicating that anoxia acted upon recovery of breaks (see introduction) — corresponding to our phase III. They based this conclusion on the supposition that the discrepancy between the two batches irradiated in air was due to a recovery process, too. This hypothesis was, in turn, based on the opinion that the males stored their sperm when not mated. This has been shown to be incorrect (LÜNING, 1952 a). When males are not allowed to mate the sperm are resorbed and/or ejaculated without copulation. For that reason, BAKER and VON HALLE's arguments in favour of the recovery process lost their cogency. Further, it has been shown (LÜNING, 1952 b) that different rates of breaks are induced in various stages of spermiogenesis, and that a greater frequency of breaks can be induced in sperm present in inseminated females than in males (LÜNING, 1954). Hence, it seems more plausible that the difference between the two sperm batches, after treatment in air, is due to difference in the rate of primary breaks rather than to differential recovery processes. By using closed-*X*

chromosomes it would perhaps be possible to decide between these two alternative explanations and to determine whether anoxia acts in phases I and II *versus* in III.

Recently, FABERGÉ (1952) and GLASS (1952) have reported that the rates of chromosome breaks induced in eggs appear to be the same as those induced in sperm with the same dose. FABERGÉ based his studies on the loss of closed-*X* chromosomes while GLASS studied the occurrence of Minute mutations. In the latter experiment there was induced 1,4 times as many Minutes in eggs as in spermatozoa. This difference, however, was not significant because of inadequate numbers of individuals.

In the present experiment yellow mutations induced in Muller-5 chromosomes were used as a test of the occurrence of minute rearrangements, and hyperploid males for gross rearrangements. In agreement with the statement of GLASS (1940, 1950) there was a low rate of gross rearrangements — a single hyperploid male was found.

On the other hand the rates of yellow mutations were high. They were, however, not constant, but decreased with increasing time from irradiation to egglaying. Expressed in terms of a percentage of the rate in the 1st and 2nd day egglaying, there was 85 % in the 3rd and 4th day and 63 % in the 5th and 6th day.

If a comparison is made between the rate of yellow mutations induced in females and that induced in males with the same dose, one must consequently take into consideration the variations in the female series which depend on the length of the egglaying period. Furthermore, one must also consider the variations in the male series which depend on the differential sensitivity of the various stages in spermiogenesis, as well as the age of the males at the time of treatment (LÜNING, 1952 b and c). The least sensitive stage in males is that sperm batch which will be used for insemination 2 or 3 days after irradiation when 0—1 day old males are used (LÜNING, 1952 a). The rate of yellow mutations under these circumstances is given in Table 2.

By comparing the rates in the female series at various times during the egglaying period (designed F 1—2, F 1—4 and F 1—6, where the figures denote the days of egg collection after irradiation) with the corresponding rates in the male series (M 2—3) significantly different variations are found in all cases, $0{,}001 > P$. The ratios are $F\ 1—2/_{M\ 2-3} = 2{,}38$, $F\ 1—4/_{M\ 2-3} = 2{,}21$ and $F\ 1—6/_{M\ 2-3} = 1{,}92$. As the rate in M 2—3 is the minimum rate, comparisons with rates received from males under other circumstances would give lower ratios than those given above. From this comparison we may conclude that there are induced about

twice as many yellow mutations in females as in males under the defined circumstances. In males it has been shown that the variations in the rates of yellow mutations agreed with the variations in the rates of breaks. A difference between the rates of yellow mutations in females and males need not indicate a difference in the rates of breaks, unless the proportion of other chromosome aberrations are the same in females and males. This is not the case with gross rearrangements, which are very rare in females, or with dominant lethals which also appear in a lower frequency in females than in males. Hence, the higher rate of yellow mutations in females than in males can not be taken as a measure of the breakability of the chromosomes in the two sexes. As the ratio between minute and gross rearrangements is much higher in females than in males, there is in females a preferential joining of broken ends from adjacent breaks, which, in relation to the rates of breaks, gives a higher ratio of minute rearrangements in females than in males.

From this we can conclude that the rate of breaks induced in females is lower than twice the rate of breaks induced in the 2nd and 3rd day sperm batch from males irradiated when 0—1 day old.

Prior to this the discussion has been confined to a comparison between irradiation of females and males in air. The effect of anoxia during irradiation of females was also studied (Tables 3 and 4). If one compares the results after irradiation in anoxia with those in air, it can be seen that there is a strong effect of anoxia (much stronger than in males), and that it affects both dominant lethals and yellow mutations to about the same degree.

If one assumes that dominant lethals induced in females are largely due to unjoined broken ends, it means that anoxia affects the number of breaks available for recombinations (phases I, II or III in Scheme 1), and probably not the rejoinability of broken ends (phase IV).

Acknowledgements. — The author wishes to express his indebtedness to Professors G. BONNIER and C. P. SWANSON for valuable discussions and to Miss E. WENSKE for technical help.

The present study was supported by the Swedish Natural Science Research Council.

SUMMARY.

(1) The present paper deals with an analysis of the effect of oxygen concentration on the rate of chromosome aberrations induced either in *Drosophila* sperm inseminated the 1st day as compared to the 2nd and 3rd day after irradiation of newly hatched males, or in oocytes.

(2) It was shown that there are induced more vital minute and gross rearrangements in sperm ready the 1st day as compared to those from the 2nd and 3rd day after irradiation in air or in nitrogen, and that in each sperm batch there are more aberrations after treatment in air than in nitrogen.

(3) The dominant lethals showed the same variation, but the second sperm batch treated in nitrogen did not show any decrease as compared to the first sperm batch.

(4) In the nitrogen series the rate of aberrations was also studied in sperm inseminated 7—10 days after irradiation. The rate was significantly higher than in those from the 1st and the 2nd and 3rd day spermatozoa.

(5) It is concluded that the variations in the breakability in various stages of spermiogenesis can not depend on variations in the oxygen concentration.

(6) In oocytes irradiated in air there are induced less dominant lethals and more minute rearrangements than in spermatozoa.

(7) The effect of anoxia is much greater in females than in males.

(8) The effect of the oxygen concentration during irradiation is discussed and a scheme of levels of possible effect is presented.

LITERATURE CITED.

1. BAKER, W. K. and VON HALLE, ELISABETH S. 1953. The basis of the oxygen effect on X-irradiated *Drosophila* sperm. — Proc. Nat. Acad. Sci. 39, 152—161.
2. BELGOVSKY, M. L. 1938. Influence of inert regions of chromosomes on the frequency of occurrence and type of changes in the adjacent active sections. — Bull. Acad. Sci. U.S.S.R. Series Biol. 5—6, 1017—1036.
3. BONNIER, G., LÜNING, K. G. and PERJE, ANN MARGRET. 1949. Studies on X-ray mutations in the white and forked loci of *Drosophila melanogaster*. II. A study of the formation of gynandromorphs and other kinds of mosaics. — Hereditas XXXV, 301—336.
4. CATSCH, A. and RADU, G. 1943. Über die Abhängigkeit der röntgeninduzierten Translokationsrate vom Reifezustand der bestrahlten Gameten bei *Drosophila melanogaster*-♂♂. — Naturwiss. 31, 368—369.

5. FABERGÉ, A. C. 1952. Loss of ring chromosomes produced by X-rays in female *Drosophila*. — Genetics 37, 579—580.
6. GILES, N. H. JR. 1952. Recent evidence on the mechanism of chromosome aberration production by ionizing radiations. — Oberlin College Symp. on Radiobiology, John Wiley and Son, Inc, N. Y. Chap, 15, 267—283.
7. GLASS, B. 1950. The effect with supplementary treatment with infra-red radiation on X-ray induced lethals and chromosome aberrations in females of *Drosophila melanogaster*. — Genetics 35, 109—110.
8. — 1952. Dosage curves for sexlinked lethals and dominant Minutes in *Drosophila melanogaster* females. — Rec. Genet. soc. Americo, 21, 28—29.
9. GLASS, H. B. 1940. Differential susceptibility of the sexes of *Drosophila* to the effect of X-rays in producing chromosome aberrations. — Genetics 25, 117.
10. KING, R. C. 1952. Reduction in productivity and recessive lethal following X-irradiation of female *Drosophila melanogaster*. — Amer. Nat. 86, 391—398.
11. LEA, D. E. and CATCHESIDE, D. G. 1945. The relation between recessive lethals, dominant lethals and chromosome aberrations in *Drosophila melanogaster*. — Journ. of Genetics 47: 10—24.
12. LÜNING, K. G. 1952 a. X-ray induced dominant lethals in different stages of spermatogenesis in *Drosophila*. — Hereditas XXXVIII, 91—107.
13. — 1952 b. X-ray induced chromosome breaks in *Drosophila melanogaster*. — Hereditas XXXVIII, 321—338.
14. — 1952 c. Studies on the origin of apparent gene mutations in *Drosophila melanogaster*. — Acta Zool. 33, 193—207.
15. — 1954. Variations in the breakability of chromosomes in mature spermatozoa of *Drosophila melanogaster* at different modes of irradiation. — Heredity 8: 211—223.
16. SCHWARTZ, D. 1952. The effect of oxygen concentration on X-ray induced chromosome breakage in maize. — Proc. Nat. Acad. Sci. 38, 490—494.
17. SIDOROV, B. N. 1936. The mutability of the *yellow, achaete* and *scute* genes in the stocks *scute-8* and *yellow 3P*. Relation between the mutation frequency of the gene and its position in the system. — Biol. Jour. 5, 3—26.
18. SONNENBLICK, B. P. Cytology and development of the embryos X-rayed adult *Drosophila melanogaster*. — Proc. Nat. Acad. Sci. 26, 573.
19. STONE, W. S., WYSS, O. and HAAS, F. 1947. The production of mutations in *Staphylococcus* by irradiation of the substrata. — Proc. Nat. Acad. Sci. 33, 59—66.
20. STRÖMNÆS, Ö. 1949. The production of dominant lethals with X-rays in aged *Drosophila melanogaster* sperm. — Genetics 34, 462—474.
21. SWANSON, C. P. 1954. The oxygen effect of chromosome breakage. — Journ. Cell. Comp. Physiol. (In press.)
22. TODAY, J. M. and READ, J. 1949. Effect of oxygen on the frequency of chromosome aberrations produced by alpha rays. — (Cited from Nature 163, 133—135.)
23. WHITING, ANNA. 1939. Sensitivity to X-rays of stage on oögenesis of *Habrobracon*. — Genetics 24, 89—90.

THE PROPHASE INDEX AND THE OCCURRENCE OF MULTIPOLAR DIVISIONS IN HUMAN CANCER CELLS

BY *EEVA THERMAN* AND *SAKARI TIMONEN*
WOMEN'S CLINIC, UNIVERSITY OF HELSINKI, FINLAND

THE cytological picture presented by a far-advanced case of human cancer is most confusing. Widely different and in part contradictory phenomena take place side by side in neighbouring cells, and at first sight it seems impossible that any cytological regularities could prevail in such a chaos. From a study of a whole series of cases, from normal tissue to highly malignant tumours, certain principles, however, emerge.

In recent years remarkably interesting results have been obtained on the cytology of the ascites tumours in the mouse and the rat (MAKINO and KANÔ, 1951; MAKINO and YOSIDA, 1951; MAKINO, 1952 a, b; BAYREUTHER, 1952; LEVAN and HAUSCHKA, 1952, 1953 a, b; LEVAN, 1954). It seems, however, too early to attempt any elaborate co-ordination of these observations and the findings in human tumours. Such a comparison remains an interesting task for the future, when more is known about both these kinds of tumours.

The present paper contains a short survey of our earlier findings concerning the mitotic mechanism in human cancer cells and the essence of our new observations together with the theory put forward to explain them.

I. THE TIMING OF THE MITOTIC PHENOMENA IN HUMAN CANCER CELLS.

Mitosis and its various modifications have been analysed by OKSALA (1944, 1953) in terms of the timing relationships of the various elementary phenomena involved. A different timing of the same elementary processes brings about widely differing results, normal mitosis grading into endomitosis on the one hand and into meiotic phenomena on the other.

It may be recalled in this connection that DARLINGTON (1948) from

purely theoretical considerations, has forecast that timing disturbances between the various cellular processes might be of importance in cancer. He says (p. 124): »In cancer I am supposing a cytoplasmic change which favours a high growth rate. In these circumstances both the nucleus and certain cytoplasmic constituents might well be unable to stand the pace.»

During our first study on human cancer (TIMONEN and THERMAN, 1950) it became evident that one of the most constant features of malignant cells consisted of a change in the ratio of metaphases to prophases. In the normal uterine epithelium this ratio is very constant and normally less than 1,5. In all cases of cancer it is increased, being, as a rule, greater than 1,5, and in many cases reaching very high values. This fact naturally indicates a corresponding change in the relative duration of the mitotic phases. The phenomenon was interpreted by us in terms of the precocity theory as formulated by OKSALA (1944). The spindle mechanism is accelerated as compared with the chromosomal mechanism, as is reflected by the shortening of the prophase. Many of the other cytological irregularities found are obviously later results of this primary change.

The idea that the multipolar divisions so frequently encountered in neoplastic tissues are due to the same cause was next presented by us (THERMAN and TIMONEN, 1950). Our conclusion may be summarized in the words (p. 401): »The origin of the multipolar divisions may also be traced back to the precocity of the extrachromosomal changes, which leads to a situation where one or both of the centrosomes divide twice while the chromosomes divide once.» The three facts on which this conclusion was based are the following: Multipolarity is by no means always connected with polyploidy. The first multipolar divisions to occur are tripolar divisions, and in addition other spindles with an uneven number of poles are also found. If multipolar divisions were to arise through the formation of restitution nuclei, quadripolar divisions ought to predominate. In regard to higher multipolar divisions, at that time we left the question open whether these might not result from restitution (p. 401): »In this way the number of centrosomes would be alternately doubled through failure of anaphase and through their precocious division, the result being the high multipolar divisions observed.»

TIMONEN (1950) carried these studies further by analysing in detail a more extensive series: 10 cases of normal endometrium, and 30 cases of cancer of the female genital tract. He reached the conclusion that the only features present in cancer but absent in the normal tissues of

his material were the change in the relative duration of prophase and metaphase, and the occurrence of multipolar divisions. The ratio metaphases/prophases was termed by him the Prophase Index. It was established that the appearance of multipolar divisions was correlated with higher values of the index.

II. THE RATIO OF METAPHASES TO PROPHASES AND THE OCCURRENCE OF MULTIPOLAR DIVISIONS.

1. OBSERVATIONS.

In the present study attention has especially been paid to the two phenomena which, according to our observations, seem to be most characteristic of human cancer cells, *i. e.* the change in the relative duration of prophase and metaphase, and the occurrence of multipolar divisions. The total number of cases examined cytologically by us is some one thousand. The Prophase Index (numerical ratio metaphases/prophases) has been determined in one hundred cases of non-malignant tissue and in the same number of cases of cancer. The cases presented in Figs. 2 and 3 thus represent a sample of the total material studied. The specimens of non-malignant cases omitted in Fig. 2 represent duplications of cases illustrated. The cases of cancer which are omitted all belong to the group with a low index. The shape of the graph is thus not changed by the leaving out of a part of the material.

From the diagnostic point of view the observations will be dealt with by TIMONEN (1954). In this connection it need only be mentioned that the increase of the Prophase Index above 1,5 is, as a rule, a fairly reliable indication of malignancy. The same is true of the occurrence of multipolar divisions. The index is further, at least roughly, positively correlated with the malignancy of the tumour, as estimated according to histological criteria.

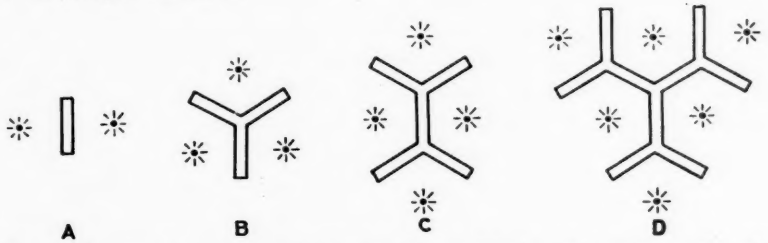


Fig. 1. Diagram of a bipolar (A), tripolar (B), quadripolar (C), and hexapolar (D) metaphase.

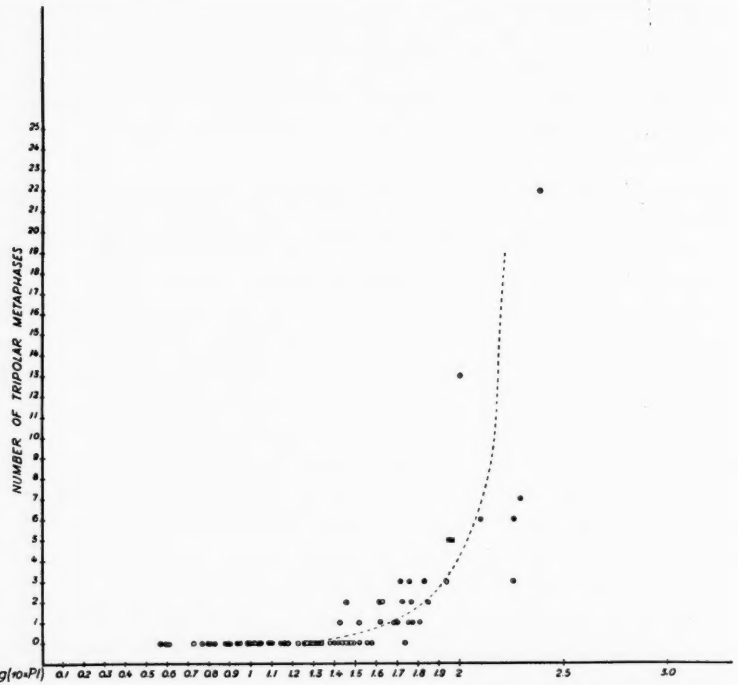


Fig. 2. Correlation of the ratio metaphases/prophases, log (10×PI), and the frequency of tripolar divisions. Each count is made from a random sample of 100 cells in prophase, metaphase, and anaphase.

In Fig. 1 a series of metaphases with different numbers of poles is illustrated diagrammatically. The cytological rules for the formation and structure of multipolar divisions have been set out in THERMAN and TIMONEN (1950). In regard to Fig. 1 it should be remembered that divisions higher than tripolar are impossible to represent adequately in a two-dimensional drawing. The other two configurations must be taken as projections of three-dimensional structures in a plane. The configurations with more than six poles are in different ways combinations of the basic structures illustrated in Fig. 1. In the following, attention has been paid to multipolar metaphases, the multipolar anaphases being disregarded. This is due to the fact that a number of multipolar metaphases disintegrate or form restitution nuclei directly from metaphase.

Fig. 2 shows the results of the analysis concerning the relationship

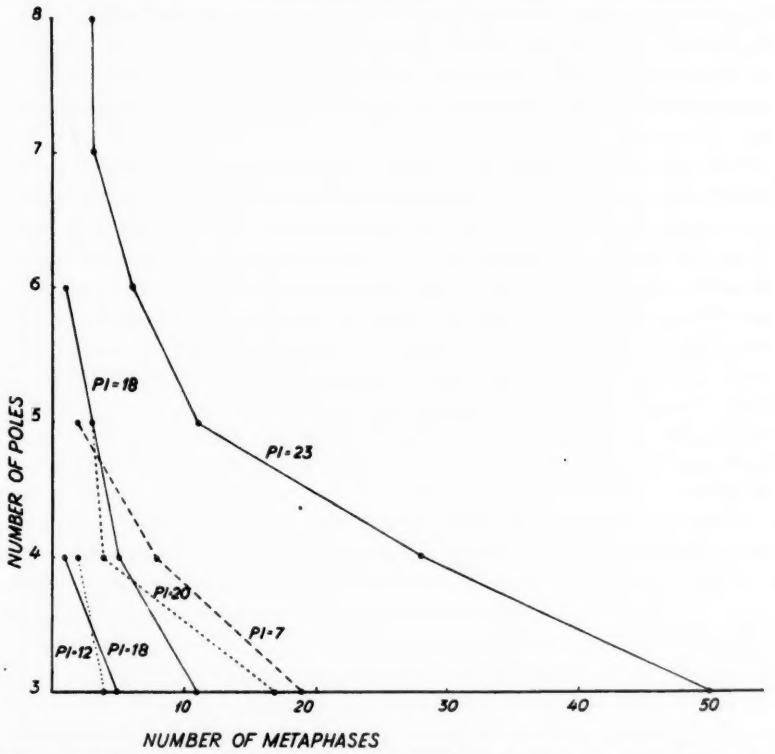


Fig. 3. Frequencies of different multipolar divisions in six cases of carcinoma as counted from a total of 200 metaphases in each case.

between the Prophase Index and the occurrence of tripolar divisions. The graph contains 20 cases of non-cancerous tissue and 48 cases of cancer of the female genital tract. The Prophase Index, *i. e.* the ratio of metaphases to prophases, has been obtained by determining the frequencies of prophases, metaphases and anaphases from a random sample of 100 dividing cells in each case. Though the index is the ratio of metaphases to prophases, anaphases were included in the count to see whether their frequency also varies. Since the frequency of anaphases seems to be fairly constant, they can obviously be left out in future counts. In the same sample of 100 cells the percentage of tripolar metaphases has been determined. In Fig. 2 the number of tripolar metaphases has been plotted against the logarithm of the Prophase Index. A clear

positive correlation is seen to exist between the Prophase Index and the occurrence and frequency of the tripolar divisions. The first tripolar divisions appear when the index reaches the value 4—6, and their percentage rises steadily reaching its highest value with the highest index found, *i. e.* 23.

In Fig. 3 the results of another count are presented. In 6 cases of cancer the frequencies of metaphases with different numbers of poles have been counted from 200 cells in each case. The frequencies of the different multipolar metaphases are shown in Fig. 3, while the bipolar divisions have been omitted. The relative number of metaphases with more poles diminishes with the increase in the number of poles. If we take as an example the case in which PI is 23, the number of metaphases with 4 poles is about one half of the number of metaphases with 3 poles; the number of metaphases with 5 poles is about one half of the number of metaphases with 4 poles, and so on. Thus, as multipolarity increases the number of metaphases decreases approximately by a factor of two.

The six cases represented in Fig. 3 were chosen as being the ones in our material which contained enough multipolar divisions to warrant a count. The counts are made from sectioned preparations except in the case PI=18 (at the right) which was made into squash preparations. Naturally a number of divisions are cut by the microtome, but since a damaged division is fairly easy to distinguish, this source of error seems to be negligible. Other difficulties are, however, involved in such a count. In configurations with a high number of poles it is often impossible to determine the number of poles exactly. In addition the configurations with a high number of poles are so rare that the figure obtained for them is inevitably subject to a great error statistically. The third factor which defies cytological analysis in cancer is necrosis, since the metabolic products of necrotic processes naturally influence the cytological picture of malignant cells.

Keeping these difficulties in mind the facts in Fig. 3 may, however, be summarized by stating that the frequencies of the different metaphases form a geometrical series:

$$a, \frac{a}{k}, \frac{a}{k^2}, \frac{a}{k^3} \cdots\cdots\cdots \frac{a}{k^n}$$

where a is the frequency of tripolar divisions and k is a constant, the value of which in most cases is near 2.

MAKINO and KANÔ (1951) have determined the percentages of differ-

ent kinds of multipolar divisions in the Yoshida sarcoma of rats, and found: 73 per cent of tripolar divisions, 22 per cent of quadripolar divisions, and 5 per cent of pentapolar divisions. These frequencies, too, form a geometrical series, although in this case k has a considerably higher value than 2.

It must be stressed that the Prophase Index naturally has significance only when normal and the malignant tissues of the same origin are compared. Thus a comparison of the index in, say, mouse and human tissues is obviously of little value. FARDON and PRINCE (1952) have obtained results which deviate from ours in that they claim that the metaphase to prophase ratios in neoplastic tissues show less variation than in normal tissues. This result may depend on several facts. Firstly, they have not compared normal and malignant tissues of the same origin. Possibly also their criteria for the determination of the mitotic stages differ from ours. The last point is made probable by their statement that an increase in the PI is found in necrotic tissues, which probably means that they have included cells showing a »colchicine-effect» in the metaphases, which we have not. Another possibility is that wandering leucocytes have been included in the metaphase count. For there is one type of these cells which seems, curiously enough, to be in a permanent metaphase or anaphase stage.

It is well-known that the percentage of dividing cells varies greatly in different regions of a tumour. Systematic determinations of the PI in different parts of one tumour are called for to determine whether or not its value varies with a varying mitotic index.

2. DISCUSSION.

The data presented in Figs. 2 and 3 show that the various cytological phenomena in human cancer cells are interrelated. The rules governing them are also partly revealed. Our observations indicate that the most constant feature in cancer is the change in the relative duration of prophase and metaphase. The technique used does not, however, reveal whether the actual change is a shortening of the prophase or a prolongation of the metaphase, or possibly both. The ultimate decision between these alternatives could naturally be made only by examining mitosis in living cells. Such observations have, indeed, been made in tissue cultures (LEWIS and LEWIS, 1932; LEWIS, 1940, 1951; MÖLLENDORFF, 1941). It must, however, be pointed out that observations made on cells in tissue culture are not, as such, applicable to cells in living

organisms. In addition, the determination of the exact length of the mitotic phases in living cells is very difficult, and it is impossible to determine exactly the beginning of prophase.

In view of this it seems that no definite evidence can be gained from *in vitro* observations for either of the alternatives, *i. e.* the shortening of prophase or the prolongation of metaphase. In many papers concerned with tissue cultures it is stated that the duration of the different mitotic phases in malignant cells is about the same as in normal cells (MÖLLENDORFF, 1941). In other papers a significant retardation of the metaphase is claimed to occur (LEWIS, 1951).

The theory, however, which tends to explain the cytological phenomena in cancer cells as resulting from the precocity of the spindle mechanism, does not postulate any absolute values for the duration of the different processes. The point at issue is, as OKSALA (1944, p. 7) has stressed in regard to meiotic precocity, *the change in the relationship of the chromosomal and spindle mechanisms in the same cell, which naturally can be brought about either by an acceleration of the spindle or by a retardation of the chromosomes.*

Other disturbances in the timing of the different processes may also, at some point, give rise to multipolarity. Thus, in the cornea of *Triturus viridescens* which was recovering after colchicine treatment, PETERS (1946) found multipolar divisions which, according to him, are to be attributed to a delay in the chromosomal cycle caused by colchicine, while the centrosomes continue to divide.

The precocity theory is most conclusively borne out by the fact that the appearance of the first tripolar divisions depends on the Prophase Index reaching a certain value, which lies between 4—6. The second proof lies in the fact that the percentage of tripolar divisions is positively correlated with the Prophase Index, rising with the latter.

OKSALA (1944, 1953) represents the extrachromosomal mechanism as causing the polarization of the cell. In the dragonflies studied by him, the polarized stage in the cell becomes gradually more and more precocious during the last premeiotic divisions when compared with the chromosomal cycle. When the precocity, as measured by a Precocity Index, reaches a certain degree, meiosis takes place. This level can be achieved either after the 11th or the 12th premeiotic mitosis.

In cancer cells, in which the cytophysiological situation naturally differs radically from that of the spermatogonial cells, the event which takes place when the precocity reaches a definite degree is not meiosis but the first tripolar division. In other words, the extrachromosomal

processes are so far advanced that one centrosome divides twice while chromosome reproduction takes place but once.

As mentioned above, the frequency of tripolar divisions rises steadily with an increasing Prophase Index. This means that the higher the precocity the greater is the probability of any particular cell becoming tripolar. In other words, every centrosome has an equal, definite probability of dividing twice, while the chromosomes reproduce but once, which probability in turn depends on the precocity of the extrachromosomal changes in the cell. Since originally all divisions in a tumour are bipolar, the probability of both the centrosomes in the same cell dividing twice is very small. This explains the fact that at first only tripolar and not quadripolar divisions make their appearance.

Only when a certain percentage of the divisions has become tripolar does a situation obtain when the simultaneous division of both the centrosomes in one cell comes within the limits of probability. When the centrosomes divide independently of each other, the occurrence of the first quadripolar division depends on the number of tripolar divisions present and the prevailing precocity. In the same way the frequency of quadripolar divisions must reach a certain value before a new precocious division »hits» one centrosome in such a cell. This naturally gives rise to the first pentapolar division. The higher multipolar divisions arise similarly stepwise from lower ones.

Bipolar divisions thus constitute the predecessors of the tripolar divisions, tripolar divisions of quadripolar divisions, and so on. And the motive force which gives rise to this process is the precocity of the extrachromosomal changes. That this line of thought is warranted is proved by the relative frequencies of the divisions with different numbers of poles, which form a geometrical series. There is no necessity to presume restitution as a significant source of multipolar divisions. The precocity of the spindle mechanism, which causes the centrosomes to divide in advance, seems to be a sufficient explanation for their origin. If restitution played an important rôle in the origin of multipolar divisions, cells with an even number of poles ought to predominate; this, however, is not the case. We have thus come more and more to regard all types of restitution as secondary phenomena which admittedly occur in malignant cells but which are not of primary importance in their cytology. In addition restitution, either from metaphase or anaphase, is not infrequently an indication of the non-viability of the cell, representing the first stages in its disintegration.

In the present paper attention has mainly been paid to the two

phenomena, the change in the relative duration of the mitotic phases and the occurrence of multipolar divisions, as being the most constant cytological phenomena in the human cancer tissues studied by us. Most of the other mitotic aberrations: laggards, stickiness of the chromosomes, so-called colchicine-effect, and others, which have repeatedly been described in human cancer (see, *e. g.*, KOLLER, 1943, 1947 a, b; TIMONEN and THERMAN, 1950; TIMONEN, 1950) vary much more in their occurrence. They are probably due, at least in part, to secondary factors, such as the growth pattern of the tumour, which in itself affects its metabolism, and to necrosis, and infection. MAKINO and KANÔ (1951) have found that these types of irregularities increase as a rat ascites tumour grows older, and its metabolism accordingly is more aberrant. It must also be remembered that most mitotic disturbances in their turn give rise to others.

Observations on the change in the duration of the mitotic phases have been made earlier by KOLLER (1947 b) who counted the frequency of the mitotic stages in one case of squamous cell cancer and one case of cervical cancer in man and compared the values with a count on spermatogonial cells in man and on ganglial cells in the grasshopper *Chortophaga*. He found that in the two cases of cancer the metaphase was greatly lengthened relative to the prophase when compared with the two kinds of normal cells. He interpreted this as showing that the nucleic acid synthesis in cancer cells must occur very rapidly during the shortened prophase. Without entering into the whole question of nucleic acid metabolism, concerning which purely cytological observations give very little information, it must be pointed out that nucleic acid synthesis, or »chromosome synthesis», as KOLLER calls it, has by no means been demonstrated to occur during prophase, which in any case would be highly improbable.

For financial aid we are greatly indebted to President J. K. Paasikivi's Fund for Cancer Research and to Sigrid Juselius' Stiftelse.

SUMMARY.

We have earlier suggested that the primary cytological change in human cancer cells is a change in the relative timing of the chromosomal and extrachromosomal processes. This change consists of an

acceleration of the spindle mechanism relative to the chromosomes, the spindle thus attaining a precocity in its cycle.

This theory was based mainly on two phenomena characteristic of cancer: The change in the relative duration of prophase and metaphase, and the occurrence of multipolar divisions.

The present observations furnish strong support for this precocity theory of cancer, and show that the various cytological phenomena are closely interrelated. The first appearance, as well as the frequency, of the tripolar divisions are clearly correlated with the ratio metaphases/prophases (Prophase Index), the value of which again seems to reflect the malignancy of the case. The frequencies of the different multipolar divisions stand in a geometrical series, *i. e.* as multipolarity increases the number of divisions decreases by a definite factor.

These facts are interpreted by us as follows: The precocity first gives rise to a change in the relative duration of the mitotic phases. Gradually a situation obtains when one centrosome in a cell divides twice while the chromosomes reproduce but once, *i. e.* the first tripolar division makes its appearance. Similarly, the first quadripolar division arises when the precocity and the frequency of tripolar divisions have reached the necessary level. In this manner the multipolarity becomes stepwise higher and higher, the lower stages furnishing the predecessors of the next higher level of division.

LITERATURE CITED.

1. BAYREUTHER, K. 1952. Der Chromosomenbestand des Ehrlich-Ascites-Tumors der Maus. — Ztschr. f. Naturforsch. 7 b: 554—557.
2. DARLINGTON, C. D. 1948. The plasmagene theory of the origin of cancer. — Brit. Journ. Cancer 2: 118—126.
3. FARDON, J. C. and PRINCE, J. E. 1952. A comparison of the ratios of metaphase to prophase in normal and neoplastic tissues. — Cancer Research 12: 793—795.
4. KOLLER, P. C. 1943. Origin of malignant tumour cells. — Nature 151: 244—246.
5. — 1947 a. Abnormal mitosis in tumours. — Brit. Journ. Cancer 1: 38—46.
6. — 1947 b. The experimental modification of nucleic acid systems in the cell. — Symp. Soc. Exp. Biol. 1: 270—290.
7. LEVAN, A. 1954. Colchicine-induced c-mitosis in two mouse ascites tumours. — Hereditas XL: 1—64.
8. LEVAN, A. and HAUSCHKA, T. S. 1952. Chromosome numbers of three mouse ascites tumours. — Hereditas XXXVIII: 251—255.
9. — and — 1953 a. Nuclear fragmentation — a normal feature of the mitotic cycle of lymphosarcoma cells. — Hereditas XXXIX (Festskrift för ARNE MÜNTZING 2.3.1953): 137—148.

10. LEVAN, A. and HAUSCHKA, T. S. 1953 b. Endomitotic reduplication mechanisms in ascites tumours of the mouse. — Journ. Nat. Cancer Inst. 14: 1—43.
11. LEWIS, M. R. and LEWIS, W. H. 1932. Malignant cells of Walker rat sarcoma No. 338. — Amer. Journ. Cancer 16: 1153—1183.
12. LEWIS, W. H. 1940. Some contributions of tissue culture to development and growth. — Growth Suppl.: 1—14.
13. — 1951. Cell division with special reference to cells in tissue cultures. — Ann. New York Acad. Sciences 51: 1287—1294.
14. MAKINO, S. A. 1952 a. A cytological study of the Yoshida sarcoma, an ascites tumor of white rats. — Chromosoma 4: 649—674.
15. — 1952 b. Cytological studies on cancer. III. The characteristics and individuality of chromosomes in tumor cells of the Yoshida sarcoma which contribute to the growth of the tumor. — Gann 43: 17—34.
16. MAKINO, S. A. and KANÔ, K. 1951. Cytological studies on cancer. II. Daily observations on the mitotic frequency and the variation of the chromosome number in tumor cells of the Yoshida sarcoma through a transplant generation. — Journ. Fac. Science Hokkaido Univ. VI, 10: 225—242.
17. MAKINO, S. A. and YOSIDA, T. H. 1951. Cytological studies on cancer. I. Morphological and statistical observations on the abnormal mitosis in tumor cells of the Yoshida sarcoma through a transplant generation. — Journ. Fac. Science Hokkaido Univ. VI, 10: 209—224.
18. MÖLLENDORFF, W. v. 1941. Zur Kenntnis der Mitose. X. Über Vorkommen pathologischer Mitosen in Gewebskulturen des Brown-Pearce Tumors. Zugleich ein Beitrag zur Frage der Tumormitosen. — Ztschr. f. Zellforsch. u. mikr. Anat. 31: 60—90.
19. OKSALA, T. 1944. Zytologische Studien an Odonaten. II. Die Entstehung der meiotischen Präkozität. — Ann. Acad. Scient. Fenn A IV, 5: 1—33.
20. — 1953. Timing relationships in mitosis and meiosis. — Proc. IX Internat. Congress Genetics (in the press).
21. PETERS, J. J. 1946. A cytological study of mitosis in the cornea of *Triturus viridescens* during recovery after colchicine treatment. — Journ. Exp. Zool. 103: 33—60.
22. THERMAN, E. and TIMONEN, S. 1950. Multipolar spindles in human cancer cells. — Hereditas XXXVI: 393—405.
23. TIMONEN, S. 1950. Mitosis in normal endometrium and genital cancer. — Acta Obstetr. Gyn. Scand. 31, Suppl. 2: 1—88.
24. — 1954. Comparison of the cytological and histological picture in cancer (in the press).
25. TIMONEN, S. and THERMAN, E. 1950. The changes in the mitotic mechanism of human cancer cells. — Cancer Research 10: 431—439.

MEIOSIS AND POLLEN MITOSIS IN X-RAYED AND UNTREATED SPIKELETS OF ELEOCHARIS PALUSTRIS

BY *ARTUR HÅKANSSON*
INSTITUTE OF GENETICS, LUND

INTRODUCTION.

RECENTLY, it was suggested by several authors that the family *Cyperaceae* has chromosomes with a »diffuse» centromere. Such chromosomes show centromere activity along their whole length, daughter chromosomes separating parallel to one another during anaphase. SCHRADER (1935) first pointed out the occurrence of this peculiar anaphase movement and used the term *diffuse centromere*. Some authors have spoken of »chromosomes lacking a localized centromere». Certain orders of insects (*Hemiptera*), Scorpions and Mites have such chromosomes (literature in LIMA-DE-FARIA, 1949). Insects having chromosomes with a diffuse centromere generally show so-called post-reduction in meiosis. The bivalents are not co-orientated at first metaphase and, thus, first anaphase is equational. Second anaphase is reductional. However, post-reduction may occur only in one sex or only in some of the bivalents (HELENIUS, 1952; SUOMALAINEN, 1953). Thus, chromosomes with a diffuse centromere may show pre-reduction.

Among Thallophytes the class *Conjugatae* seems to possess chromosomes with a diffuse centromere (*Spirogyra*, GEITLER, 1930; Desmids, KING, 1953). Important was the discovery that the angiospermous genus *Luzula* has this kind of chromosomes. The Portuguese cytologists CAMARA, DE CASTRO, MALHEIROS, and GARDÉ (1947—1951) discovered and studied them in *L. purpurea*, a species with few ($2n=6$), relatively large chromosomes. This species was also studied by ÖSTERGREN (1949). The chromosomes are thick and show no constrictions. The Portuguese authors often stress the fluid nature of the matrix of the chromosomes, making them more sticky than ordinary chromosomes. At metaphase the chromosomes lie flat with their whole length in the equatorial plate, at anaphase daughter chromosomes move apart parallel to each other. During the anaphase movement the two ends of a daughter chromosome are bent towards the pole, whence it seems concave.

The course of meiosis in *L. purpurea* was shown to be similar to that in most animals having chromosomes with a diffuse centromere. The first division was equational, the second reductional (MALHEIROS, DE CASTRO, CAMARA, 1947). This was later confirmed by LA COUR (1953).

Very striking is the behaviour after x-raying of chromosomes with a diffuse centromere. HUGHES-SCHRADER and RIS (1942) x-rayed a Coccid. As usual the irradiation caused breakage and thus increased the number of chromosomes, but the fragments behaved as ordinary chromosomes undergoing normal mitosis, proving that they were not acentric. This was the *experimentum crucis* which proved the existence of a diffuse centromere. DE CASTRO, CAMARA and MALHEIROS (1949) showed that plants from x-rayed seeds of *Luzula purpurea* had fragments which divided at anaphase like ordinary chromosomes. Only very small fragments, so-called »minutes», were lost; they were interpreted as small parts of chromosome matrix. LA COUR (*l. c.*), who irradiated inflorescences of *L. purpurea*, confirmed the observations of DE CASTRO *et al.* Lost minutes, however, were interpreted by him as acentric fragments. LA COUR considers the chromosomes of *L. purpurea* to be polycentric rather than diffuse-centromeric; two breakages between neighbouring centromeres would then produce an acentric fragment.

Most *Luzula* species have rather small chromosomes but no doubt a diffuse centromere characterizes the whole genus (see also NORDENSKIÖLD, 1951; MICHALSKA, 1953) and probably the whole family *Juncaceae*. Only chromosomes which are not too small can be studied sufficiently in detail to allow an analysis of the centromere conditions; this is probably the reason why we have no knowledge of diffuse centromeres in *Juncus*.

It seemed that *Eleocharis palustris* subsp. *vulgaris* ought to be a suitable representative of the famliy *Cyperaceae* in an attempt to elucidate the centromere conditions in this family. I have investigated this form earlier (HÅKANSSON, 1928, 1929). The chromosome number is $n=19$. The chromosomes show distinct size differences, and may be divided into three classes, large (L), medium (M) and small (S), there being 3 L, 5 M and 11 S. The size differences are most distinct in pollen mitosis, and in reality there were still more size classes, L, M as well as S chromosomes having different sizes. New observations have now been made on root mitosis and pollen mitosis, and meiosis has for the first time been studied in this subspecies. Meiosis and pollen mitosis have also been studied in spikelets which were x-rayed one minute with 500 *r*, 750 *r* or 1000 *r*, respectively, and fixed after 48 or 72 hours.

MITOSIS IN ROOTS.

In an earlier publication it was stressed that the chromosomes are closely packed in the plates and difficult to study. New observations on longitudinal sections show that the appearance and behaviour of the chromosomes at metaphase and anaphase are similar to those described from *Luzula* mitosis. The large chromosomes were rather favourable to study. The chromosomes are rather thick and show no constrictions. At pro-metaphase they are often bent (Fig. 1), at metaphase they lie flat in the plate. At early anaphase daughter chromosomes separate parallel to each other, the two ends being bent towards the pole (Fig. 2). At late anaphase the chromosomes are more strongly bent. The spindle is, as in *Luzula*, very broad and truncate. However, in *Eleocharis* the ends of anaphase chromosomes are sometimes not bent: the concavity of the chromosomes is less pronounced or may not appear at all. This may depend on variations in the form of the spindle.

At prophase the chromosomes showed differentiation. An earlier stage showed coiled chromosomes. Several satellites were observed in the prophase nucleus. The chromosomes often had one or several segments that were unstained or only slightly stained. Smaller chromosomes sometimes had strongly stained end-segments, whereas the middle part of the chromosome was more weakly stained. Other chromosomes may show two such slightly stained segments. The large number of chromosomes make a close study difficult.

As was previously described, *E. palustris* plants with $n=19$ have, on an average, more nucleoli than plants with $n=8$. After nucleolar stainings 1—6 nucleoli were observed in the present material. It seems that a nucleolus is formed from four or sometimes from six (five) chromosomes. Nucleoli may later unite.

FIRST POLLEN MITOSIS.

In *Cyperaceae* the four tetrad nuclei after meiosis gradually take an excentric position near the inner end of the pollen mother cell. They locate themselves tetrahedrically with one nucleus directed outwards. The latter increases in size while the inner nuclei remain small. Wall formation is strongly delayed in the tetrad. First at the time of pollen mitosis the small nuclei are included in separate cells, but the separating membranes may be evanescent. Mitosis begins simultaneously in all four nuclei, but only the large nucleus undergoes anaphase. In the small nuclei chromosomes have a subnormal size. The membrane of a small

1
2
3
4
5
6
7
8
9
10
11
12 a
12 b
13
14
15
16
17

nucleus may disappear and a normal or irregular metaphase plate be formed, then telophase sets in and a new nucleus with a very dense structure is formed. Once or twice an irregular anaphase was observed in a small cell (Fig. 7). The irregularity was perhaps due to the fact that lack of space had prevented the forming of a normal spindle. The generative cell is formed near the small tetrad nuclei, the spindle being longitudinal. In *Cyperaceae* its formation shows certain peculiarities which have been described several times. Judging from a photomicrograph published by ÖSTERGREN (*l. c.*, Fig. 19) this process is similar in *Luzula* and in *Eleocharis*. In the latter genus, however, the pollen nucleus is large and favourable to a study of the chromosomes.

Early prophase shows split chromosomes which often are bent; sometimes the ends of a chromosome meet to form a ring. A few times two satellites were observed (Fig. 3). At late prophase chromosomes were heavily charged with nucleic acid, splits and satellites were invisible. At metaphase the chromosomes are thick and usually straight. They lie flat in the plate and a parallel separation of daughter chromosomes could be observed at early anaphase (Figs. 4 and 5). Polar views often show the anaphase plates as replicas of the metaphase plates (Fig. 6). Thus, there is evidence of a diffuse centromere also in pollen anaphase, but the chromosome ends are usually only weakly or not at all bent to the pole. Often the chromosomes take an oblique position in the spindle during anaphase.

The investigation of the irradiated spikelets showed that 1000 *r* had been to strong a dosage. As a rule, mitosis was destroyed which implies that the nuclear membrane had disappeared, but the chromosomes had not been able to form a spindle or a metaphase plate: they formed an irregular, rather scattered gathering in the cytoplasm. A

1: root mitosis, chromosomes at pro-metaphase. ×2000. — 2: root mitosis, early anaphase. ×2000. — 3—17: first pollen mitosis. — 3: prophase, two satellites. ×1500. — 4: early anaphase. ×1500. — 5: early anaphase, several bundles of spindle fibers attached to one chromosome. ×2000. — 6: the anaphase plates, the »generative» plate below. ×1500. — 7: anaphase of a small tetrad nucleus; the large cell shows telophase, the upper nucleus being bent, is incomplete. ×1500. — 8: prophase, small fragments and chromosome fusion. 750/48. ×2000. — 9: prophase, increase of chromosome number through breakage. 750/48. ×2000. — 10: metaphase plate, 21 chromosomes and two fragments; the spindle was here transverse. 500/72. ×2000. — 11: anaphase, chromosomes lagging. 500/45. ×1500. — 12 *a*: Ditto. ×2000. — 12 *b*: delayed division of two chromosomes. ×2000. — 13: tube nucleus and generative nucleus in close contact. 500/48. ×1500. — 14: restitution nucleus in the pollen grain. 500/48. ×1500. — 15: supernumerary »tube nucleus». 500/48. × 1500. — 16: triploid plate formed of the small tetrad nuclei. ×2000. — 17: union of plates with larger and with smaller chromosomes. — ×2000.

similar damage may sometimes be observed after 500 *r* and 750 *r*. In the following description such cases will be ignored.

The prophase nuclei often show changed chromosomes. After 750 *r* the pollen nucleus may contain some small fragments but also fragments having the size of chromosomes. It turned out a disadvantage that the chromosomes of *E. palustris* have different sizes; this makes a direct establishment of structural changes difficult. The nucleus in Fig. 8, has, however, a very large chromosome which proves to be a structural change, probably caused by fusion. Increased numbers of chromosomes and the occurrence of very small fragments were more frequent after 750 *r* than after 500 *r*. Very often, however, irradiated nuclei had only 19 chromosomes. The increase in chromosome number may be moderate or large, to 20—23 or to 28—30 chromosomes, respectively (Fig. 9).

Metaphase and anaphase stages are best studied in Feulgen-stained slides. The most changed prophase nuclei do not seem to undergo mitosis or to form a regular plate. Otherwise the chromosomes form a metaphase plate; in this plate also rather small fragments may be observed. Fig. 10 shows 21—22 chromosomes, one small and one minute fragment. Only rarely chromosomes and fragments outside the plate are observed. Large chromosomes sometimes lay parallel to the spindle. Anaphase was often disturbed, showing bridge formation and lagging. A large chromosome may be lagging between the anaphase groups and is in such cases always in the direction of the spindle fibers (Fig. 11). The chromosome is no doubt pulled to different poles, owing to breakage and reunion (compare p. 343). There are often several bridges; perhaps all bridges are not an indication of structural change, some may be due to increased stickiness. Several lagging chromosomes may be observed; they have divided but the anaphase movement is delayed (Fig. 12). Division of lagging minute fragments was not observed, but in most cases they seem to be included in a telophase nucleus. The distance between the two telophase groups is, however, usually very small in x-rayed pollen mitosis.

The including of supernumerary elements of fragment size in a plate speaks in favour of the hypothesis that the chromosomes have a diffuse centromere or several centromeres. In untreated material pollen mitosis with 20 instead of 19 chromosomes has been observed in rare cases. The supernumerary element was here an unchanged chromosome, but the occurrence of centric fragments in the x-rayed material can hardly be doubted. Direct observation of the attachment of the

spindle to the chromosome is difficult. The fibrillar structure of the spindle may be very clear, but it seemed very susceptible to the fixing fluids. Fig. 5 shows early anaphase; bundles of fibres were attached to the chromosomes, several bundles to one chromosome. As in *Luzula*, separating chromosomes show some stickiness at the earliest stage of separation.

The spindle is susceptible to irradiation. In the treated spikelets the spindle of the pollen mitosis was, as a rule, more broad than under normal conditions; at late anaphase the spindle in most cases did not undergo the usual elongation. The result is that the tube nucleus and the generative nucleus lie close together (Fig. 13). The former is as usual larger and less stainable. In other cases the nuclei were at some distance from each other but the irregular anaphase may leave a bridge between them. The nuclei were sometimes more or less united; their difference in structure disappeared when the nuclei became more intimately connected; a restitution nucleus of uniform structure may be formed (Fig. 14). On the other hand, the irregular pollen mitosis may lead to the formation of more than two nuclei, as many as five were observed. Fig. 15 shows two tube nuclei and one generative nucleus. Usually only one of the nuclei had a »generative appearance», but once two generative nuclei lay side by side at the pole. Thus, supernumerary nuclei are formed because the chromosomes are rather scattered after anaphase, particularly at the vegetative pole of the spindle. Probably supernumerary nuclei in pollen grains may also be produced directly from scattered pro-metaphase chromosomes due to absence of the spindle, as assumed by KOLLER (1943) concerning irradiated *Tradescantia*. Aberrant orientation was another spindle defect observed, the spindle in rare cases being transverse (compare Fig. 10). In such cases no generative cell is formed, only two nuclei of uniform appearance. This is a pollen anomaly that has been described many times; it has, for instance, been observed in species hybrids and in plants with a relatively high number of accessory chromosomes.

The high dosage 1000 *r* often killed all nuclei of the pollen tetrad, no further development occurring. After 500 *r* and 750 *r* the three small tetrad nuclei may take a dense structure very rapidly before the pollen metaphase sets in, but may in other cases be more vigorous than usual and show slightly increased size. Their behaviour is uniform within the same grain. Enlarged tetrad nuclei usually were not in separate cells, they had often moved from the inner end of the grain to a position more near the centre. The formation of a separate generative cell may

also be inhibited, the nucleus may remain free and lack cytoplasm of its own.

In some spikelets, all probably belonging to the same clone, a peculiar irregularity was observed during pollen mitosis. The non-functioning tetrad nuclei formed comparatively large metaphase plates with comparatively large chromosomes. Two or three small nuclei often formed a diploid or triploid plate or pro-metaphase (Fig. 16). Sometimes the normal pollen nucleus was polyploid: one grain had only one small tetrad nucleus and a large and, as it seemed, triploid spindle. Such cases may be the result of irregular meiosis; union of nuclei was observed at the second telophase. Sometimes, however, the normal plate with large chromosomes was continuous with a plate formed from a small tetrad nucleus (Fig. 17). Thus it seems as if chromosomes from different tetrad nuclei may be brought together, and also that only a part of the chromosomes from a small nucleus may be included in the large plate. A plate with 34 chromosomes may be explained in this way.

SECOND POLLEN MITOSIS.

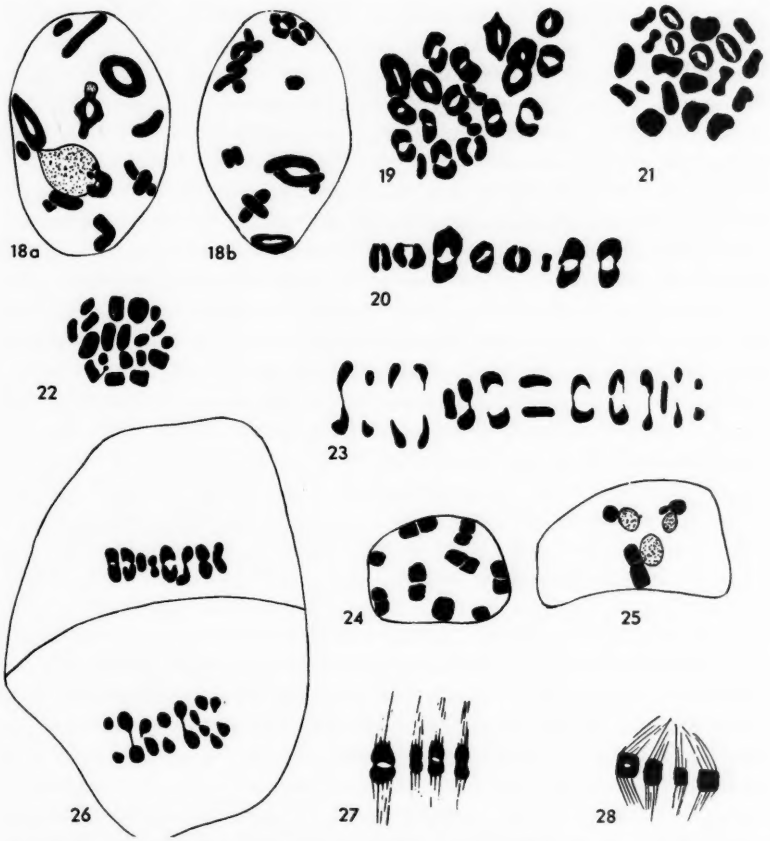
The nuclei of old pollen grains seemed very resistant to x-raying. In some spikelets x-rayed with 1000 *r* and fixed after 72 hours second pollen mitosis was found and seemed perfectly normal. The spindle was long and attenuated, no lagging or bridge formation was observed at anaphase. The small diameter of the elongated generative cell and of the second spindle made exact counts of the chromosomes impossible. Prophase nuclei had about 19 chromosomes.

It is probable that the increased thickness of the exine of the pollen grain and, above all, the accumulation of nutrients within the grains — which had now a dense content — are the causes of the higher resistance.

MEIOSIS.

It seems to be very easy to obtain good plates of first metaphase in *Cyperaceae* but the structure of individual bivalents is less distinct. The bivalents are very contracted and thick; at diakinesis they are often in contact. Meiosis in *Eleocharis palustris* has previously been studied by PIECH (1924), who investigated the subspecies with $n=8$.

Diakinesis in a macrospore mother cell is shown in Figs. 18 *a* and *b*. It is a rather early stage, the nucleolus still being present. Several bivalents have the form of a ring, others have only one chiasma. Interstitial



18 *a* and *b*: diakinesis in a macrospore mother cell. ×2000. — 19: pro-metaphase. IV?+17 II+fr. ×2000. — 20: some bivalents from a metaphase plate. ×2000. — 21: first metaphase, polar view, 19 II+fr. ×2000. — 22: 19 II, satellite. ×1500. — 23: early anaphase, all bivalents were not distinct. ×1500. — 24: interkinesis nucleus. ×1500. — 25: interkinesis nucleus, only nucleolus forming chromosomes are pictured. ×2000. — 26: second metaphase in an ovule. ×1500. — 27: several spindle fibers are attached to the chromosomes at early second metaphase. ×2500. — 28: anomalous spindle with »rifts». ×1500.

chiasmata may be formed, one large bivalent has three. Three bivalents are in contact with the nucleolus and have a different appearance; one carries distinct satellites. Later on the bivalents are strongly contracted.

The bivalents at pro-metaphase and metaphase were most distinct

in the slides of a spikelet which had a supernumerary fragment chromosome. Ring-bivalents were very common here. The pro-metaphase in Fig. 19 had probably been slightly squashed, making all the bivalents distinct. It is uncertain whether the largest element is a quadrivalent or two interlocking bivalents. In *Luzula purpurea* the ring-bivalents at metaphase lie transversally to the spindle in the equatorial plate. Such an orientation is no doubt rare in *Eleocharis*. Fig. 20 shows some bivalents at early metaphase, Fig. 21 a polar view of the metaphase plate. The longitudinal orientation of ring-bivalents is clear in this plant, although the direction of the individual bivalent seems to vary.

As a rule the structure of the bivalents is, however, impossible to ascertain. The plate usually contains 19 bivalents. Fig. 22 shows that in rare cases satellites may be visible — also observed in the macrospore mother cell. Exceptional plates had 20 elements, no doubt 18 II+2 I. Multivalents were probably very rare (bivalents in contact may simulate multivalents). This was surprising, because this form of *E. palustris* shows much evidence of autopolyploidy and it is known that in autopolyploid forms of different *Carex* species multivalents are common. An early anaphase stage is shown in Fig. 23. It is uncertain, however, if this is a typical picture of this stage, since it is rare and usually indistinct.

Carex bivalents in the metaphase plates pictured by WAHL and TANAKA often have a distinct double structure. In polar plates of *Eleocharis* such bivalents may also be observed (compare Fig. 21): four bivalents seem to have a median constriction. The chromosomes of the interkinesis nuclei generally are double, having a median split. The chromosomes retain their double structure rather unchanged during interkinesis (Fig. 24), but nucleoli appear on some chromosomes. PIECH stated that no nucleoli are formed during interkinesis, but stainings kindly performed by Mr. J. REITALU clearly showed a small nucleolus on two or three chromosomes of different size (Fig. 25). The nucleoli remain attached to the chromosomes, but in the cytoplasm appear one or two spherical bodies stainable with light-green. These bodies are no longer visible at the beginning of the second division, but new extranuclear bodies appear at second telophase.

The cytoplasm of the pollen mother cell undergoes certain changes during interkinesis. Sometimes a circle or plate of minute bodies was visible for a short time on each side of the equator. It seemed that here the formation of a cell plate was initiated but it was never completed and was of an ephemeral nature. As is well known, the pollen formation

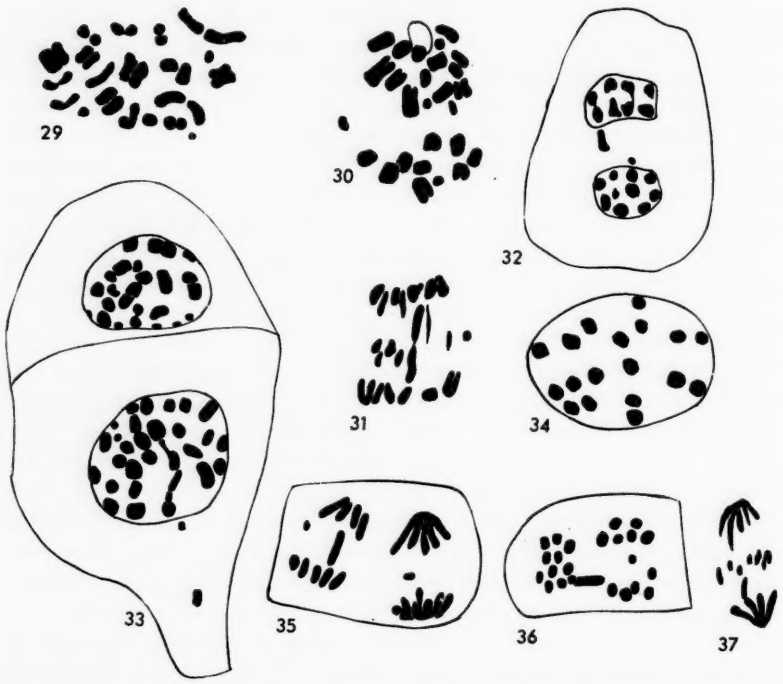
in *Cyperaceae* is according to the simultaneous type. However, WULFF (1939) considered it to be a reduced form of the successive type, referring to an old observation of JUEL of an ephemeral cell plate at interkinesis in *Carex acuta*. The changes in the cytoplasm observed in *Eleocharis* seem reminiscent of a successive pollen formation.

Interkinesis in *Eleocharis* is similar to the same stage in *Carex*, as shown in a photomicrograph by WAHL (*l. c.*, Fig. 61). WAHL called the chromosomes pairs, that is, they were considered to be half-bivalents, an interpretation which seems uncertain in *Eleocharis*. At second metaphase they seem orientated with the split in the equatorial plane, but this stage was not very distinct. Fig. 26 shows the second division in the ovule.

In x-rayed spikelets meiosis was more or less often disturbed. The most common irregularities were increased number of chromosomes in the plates, the appearance of very small fragments, lagging and bridge formation at first and second anaphase. The second anaphase was more disturbed than the first.

Supernumerary chromosomes at first metaphase are no doubt often univalents. Different investigators have shown that in *Cyperaceae* univalents are in the equatorial plate, divide, chromatids passing to the poles with the other chromosomes. The plate may also contain fragments (Fig. 29). In most cases the plate contains all the chromosomes and fragments, but elements may sometimes be observed outside the plate. The orientation of the bivalents seems variable; sometimes one, sometimes both of the paired chromosomes are seen in polar view (Fig. 29). A few times an amphibivalent was observed; it was placed in the longitudinal direction of the spindle. After treatment with 750 *r* the bivalents may be eroded, after 1000 *r* fusion of the bivalents to a large ring in the periphery of the plate may be observed (compare DE CASTRO, CAMARA, and MALHEIROS, 1949).

The behaviour of small fragments at first anaphase is shown in Fig. 30. The cut of the knife has hit the spindle obliquely and only a part of the lower plate is in the pictured section. There are fragments at each plate, while one fragment lags at the equator of the spindle. At late anaphase lagging chromosomes and fragments may occur; loss is less frequent although it may be observed (Fig. 32). The division of small fragments is rarely observed but undivided fragments seem to move to the pole. Bridge formation may also occur at first anaphase after 750 *r* (Fig. 31), a bridge connecting the two interkinesis nuclei was also observed. The interkinesis stage had no uniform appearance. It was



29: metaphase 1; a minute segment, indicated through a ring, was not in the plate. 19 II+3 fr. 500/72. ×2000. — 30: first anaphase; fragments in the anaphase groups, one fragment lags at the equator. 750/48. ×2000. — 31: very irregular first anaphase 750/48. ×2000. — 32: interkinesis, one large chromosome and one fragment are »eliminated». 750/48. ×1500. — 33: interkinesis in the ovule; all chromosomes in the nuclei are not pictured, two »eliminated» fragments. 1000/72. ×1500. — 34: interkinesis nucleus, the chromosomes are simple. 500/48. ×1500. — 35: second anaphase. 500/48. ×1500. — 36: Ditto. — 37: the second division, eight fragments lagging at the equator. 750/48. ×1500.

perhaps some defect of the spindle — which prevented an assembling or congression of the chromosomes in an anaphase group — that caused the forming of very large interkinesis nuclei. These nuclei apparently had an increased number of chromosomes, but it was only a separation of chromosome halves along the split (Fig. 34). Spikelets irradiated with a higher dosage may show fragments in the interkinesis nucleus; Fig. 33 shows such an interkinesis in an ovule. In other cases again interkinesis nuclei had normal structure.

After 500 *r* the second meiotic division shows lagging and bridges

in less than 4 % of the pollen mother cells (Figs. 35 and 36). In Fig. 36 a large chromosome, »eliminated» at the first division, unites anaphase groups from different spindles. This was one of the very few cases observed which indicated loss at the first division at this dosage. After 750 *r* the second division was very often irregular. Frequently half a dozen or more fragments lag at the equator (Fig. 37) or lie between the equator and the pole. A median segment of some chromosomes seemed sometimes to be left at the equator. Large elements may be divided, the small fragments are, however, always single. Bridges are very frequent. There is only small evidence of these irregularities in the young tetrads, however.

Spindle disturbances were not so frequent at meiosis as at first pollen mitosis. The normal spindle at first metaphase seems very similar to this spindle in *Luzula campestris* (judging from photomicrographs in BROWN, 1950). In *Eleocharis palustris* the attachment of several spindle fibers to one chromosome was observed, as is seen in Fig. 27, which shows early metaphase of the second division. BROWN described certain peculiarities of the *Luzula* spindle. One was the appearance of »rifts», which he considered as an artefact caused by fixative or smearing. Such »rifts» were sometimes observed here (Fig. 28) in some slides of x-rayed spikelets which had a heavily stained spindle at first metaphase; occasionally the spindle was divided longitudinally, causing »rifts».

A peculiar case of an aberrant spindle was once observed after 500 *r*. The bivalents were all in the periphery of the pollen mother cell, each attached to some distinct spindle fibers. All fibers of the spindle united in the centre of the cell. Thus a unipolar spindle was formed, shaped as a wheel with the fibers as spokes uniting at the centre (the wheel-nave), the bivalents being in the periphery. Thus there were only spindle fibers on one side of the bivalent, and anaphase separation seemed improbable. The other pollen mother cells of the loculus showed a more advanced stage, interkinesis or second anaphase.

CONCLUSIONS.

The evidence of a diffuse centromere in *Cyperaceae* has hitherto been of an indirect nature. Attention has been called to a close affinity between *Cyperaceae* and *Juncaceae*, to the many aneuploid chromosome numbers and to the occurrence of post-reduction in *Carex* (described by WAHL, 1940).

Cyperaceae has a very peculiar pollen development, but WULFF

(1939) has shown several peculiarities in the pollen tetrads of *Juncaceae* suggestive of *Cyperaceae*. His conclusion »dass vom embryologischen Standpunkt aus eine nahe systematische Verwandtschaft zwischen den beiden Familien als bewiesen angesehen werden kann», seems correct. Earlier, *Juncaceae* was often placed in the order *Liliiflorae*.

The chromosome numbers are, as a matter of fact, very different in *Juncaceae* and *Cyperaceae*. The species of the former family have euploid numbers and aneuploidy is rare. The genus *Luzula* has 3 as basic number, the multiple numbers are assumed to be caused by a transverse division of the chromosomes, a process called agmatoploidy by MALHEIROS-GARDÉ and GARDÉ (1951). Polyploids arisen through agmatoploidy (endo-nuclear polyploidy, NORDENSKIÖLD, 1951) have the same number of nucleoli and SAT-chromosomes as the diploid and much smaller chromosomes. Diffuse centromeres make such fragmentations possible, although it may seem strange that all chromosomes have been divided, and, as it seems, in two equal parts. The theory is supported, however, by the observation of NORDENSKIÖLD that in certain hybrids several small and one large chromosome are paired. Aneuploidy in *Luzula* may originate from fragmentation of one or a few chromosomes; a further result in such cases are chromosomes of different size within the same set. A few cases of true polyploidy are also known in *Luzula*. The genus *Juncus* has the basic number 5; the lowest haploid number actually found is 15.

Cyperaceae is, on the other hand, distinguished through aneuploid chromosome numbers. In *Carex* many species have chromosomes of rather equal size, others show considerable size differences within the set. At first the complete absence of polyploidy in *Carex* was advocated, but later several cases of autopolyploidy were found, for instance *C. siderosticta* with $n=6$ and $n=12$ (TANAKA, 1940). In quite a number of species different chromosome numbers have been established; there are races with multiple numbers or aneuploid numbers. TANAKA (1949, p. 22) summarizes his investigations thus, »Recently the author has reported that up to the present a varying number of from two or more to as many as six, intraspecific aneuploid chromosome numbers were found in some seventy species, and that various degrees of the structural hybridities were noted in fifty-three species, and that only fifty-nine species out of 149 species studied were recognized as a stable species». The trend in the new investigations (WAHL, 1940; TANAKA, 1949) is to assume the original existence of several polyploid series within the genus, probably with different basic numbers. The aneuploid

species are derived secondarily through disturbed meiosis or through crosses between aneuploid plants with a high degree of polyploidy (TANAKA, *l. c.*). Structural changes are also frequent. So far, the chromosome changes have not been discussed in the light of the possibility that the *Carex* chromosomes have a diffuse centromere. Agmatoploidy may explain different sizes within the set and, as in *Luzula*, the small chromosome size in many polyploids is striking. In *Scirpus s. l.* we also find aneuploidy to be very frequent; often the chromosomes are small in species having a high number, and there are often different numbers in the same species. Different chromosome sizes within the same set are particularly frequent in *Eleocharis*.

Eleocharis has large chromosomes, »agmatopolyploidy» does not occur here. On the contrary, in *E. palustris* forms with a higher chromosome number have, on an average, also larger chromosomes. The basic number seems to be 5, it occurs in *E. parvulus* and *E. obtusa*. LEWITSKY (1940) found it in a dwarf form of *E. palustris* from the vicinity of Kiew, »growing far back from the river on clayey waste lands». In Sweden *E. palustris* with $n=8$ and $n=19$ has been found (HÅKANSSON, 1929). They had different sizes within the chromosome set, the latter 3 L + 5 M + 11 S, the former smaller chromosomes with 2 L, probably 1 M, and 5 S. This is also the chromosomes found in 8-chromosome plants from Massachusetts, U.S.A., by HICKS (1929). Plants with 8 and 19 chromosomes may be very similar but show differences in fruit size (HÅKANSSON, *l. c.*). WALTERS (1949) has given these two chromosome races in *E. palustris* the rank of subspecies. Subsp. *vulgaris* ($2n=38$) has larger fruits, larger stomata and a more northern distribution than subsp. *microcarpa* ($2n=16$). The chromosome numbers of English plants of *E. palustris* have been determined by DOXEY (1938) and WALTERS. The former found a certain variation, »$2n=38$ and $2n=39$ for several *E. palustris* plants in northern English localities, also single plants with $2n=37$ and 40—41» (cited after WALTERS). HICKS has also found the chromosome number $n=18$. Stout plants called var. »*glaucescens*» had this number; 5 of the chromosomes were large, 13 smaller and of equal size. *E. palustris* with $n=8$ has also been reported from Poland, Hungary and Russia.

Thus, in addition to *E. palustris* subsp. *microcarpa* and *E. palustris* subsp. *vulgaris* (the latter is called subsp. *palustris* in the new »Flora of the British Isles») we have in Europe the dwarf form found by LEWITSKY. Comparing the latter with *E. palustris* with $n=8$, LEWITSKY writes, »In general appearance the chromosomes of both forms are very similar,

differing slightly only in size, so there are no grounds for presuming segmentation or association of chromosomes. If we assume the common form is the initial one three pairs of chromosomes must have been eliminated». However, when it has now been shown that the chromosomes have a diffuse centromere, the theory that the number 19 has arisen through doubling of 8 and fragmentation of at least one large chromosome seems more plausible (see HÅKANSSON, 1929). The dwarf form perhaps shows the original chromosome conditions whereas *microcarpa* and *vulgaris* are on the tetraploid and hexaploid levels, respectively. The occurrence of three nucleolus-forming chromosomes of at least two sizes in subsp. *vulgaris* is further evidence of polyploidy and fragmentation. It was pointed out in the previous paper that the size differences within the set were less striking in root mitosis and in the 8-chromosome form and, further, that the general chromosome size was smaller in the latter form. The first two points may explain why LEWITSKY, who studied root mitosis, does not describe size differences in his 8-chromosome plants; they are, however, rather clear in the pictured plate whereas in the plate from the dwarf form no differences are visible. In conformity with the third point the dwarf has smaller chromosomes. The absence or rarity of multivalents in subsp. *vulgaris* is unexpected and indicates alloploidy and larger differences than may be shown by the very variable phenotypes.

Very closely related to *E. palustris* is *E. uniglumis* (WALTERS, *l. c.*). The chromosomes are very similar to those of subsp. *vulgaris* but their number is higher, $n=23$ (HÅKANSSON, 1928). The same number has been found in British plants from different localities. From the continent, however, the haploid numbers 16 (PIECH) and 8 (PFEIFFER) have been reported. The genus *Eleocharis* is distinguished by comparatively large chromosomes of different size. Different intraspecific numbers have also been reported in *E. acicularis*.

These different aneuploid, rather deviating intraspecific chromosome numbers in *Eleocharis* and the unequal size of the chromosomes within the set strongly suggest that fragmentation and perhaps also fusion have contributed to the chromosome variation in *Cyperaceae*, in addition to the more familiar changes: autoploidy and true aneuploidy through meiotic irregularities or crossings. Formation of new chromosomes through fragmentation is only possible in chromosomes with several centromeres or a diffuse centromere. There is also evidence of fusion of chromosomes in *Scirpus s. l. S. lacustris* (*Schenoplectus lac.*) and the related *S. Tabernaemontani* have 21 chromosomes of equal size.

In addition, TANAKA has found two forms with $2n=40$ (var. *pictus*) and $2n=38$, respectively. The former had one, the latter two very large chromosomes. This large chromosome had the size of three ordinary chromosomes and in meiosis may pair with three other chromosomes. Thus, it was no doubt composed of three chromosomes and its origin through simple fusion now seems a possibility. TANAKA (1939), who, of course, assumed the chromosomes to be monocentric applied »the conception of fragmentation and fusion in NAVASHIN's sense» to the origin of the large chromosome; a rather complicated procedure was suggested.

The appearance and behaviour of the chromosomes of *Eleocharis palustris* show very strong similarity to those described from mitosis in *Luzula purpurea*, indicating polycentric chromosomes or a diffuse centromere. The peculiar parallel separation of daughter chromosomes and the concave form of the anaphase chromosome — simulating an attraction of the two ends to the pole and also very distinct in Scorpions, Coccids and certain other animals with a diffuse centromere and fairly large chromosomes — were observed here. Satellites are difficult to demonstrate in *Eleocharis palustris* and other *Cyperaceae* as well as in *Luzula*, although perhaps they are present in all species. TANAKA (1940), however, reported one and two satellites, respectively, in the sets of diploid ($2n=6$) and tetraploid ($2n=12$) *Carex siderosticta*. The haploid set of *E. palustris* has two or perhaps three satellites, only occasionally observed. Recently a small satellite has been observed in *Luzula purpurea* and *L. campestris* (see a note in MALHEIROS-GARDÉ and GARDÉ, 1951), in the latter species BROWN (1951) described a large heterochromatic, terminal centromere as nucleolus-organizer at pachytene. Perhaps the thread carrying the satellites is, as a rule, very short.

The spindle in *Eleocharis* and in *Luzula* is broad and truncate. Extreme in these respects was the spindle of the first meiotic division in *Eriophorum vaginatum* (HÅKANSSON, 1928), another member of the family *Cyperaceae*. In *Eleocharis palustris* the spindle is sometimes pointed in meiosis or first pollen mitosis, the second pollen mitosis always showed a long, thin spindle. The attachment of several spindle fibers or bundles of spindle fibers to one chromosome was observed, although the observations hardly allow conclusions as to the question of a diffuse centromere or polycentric chromosomes.

The occurrence of post-reduction in *Carex* has been an argument in favour of a diffuse centromere. Post-reduction was, however, described by WAHL (1940) only. His interpretations are based on the observation that associated chromosomes, that is bivalents, trivalents, chromosome

rings, *etc.*, remain together at early first anaphase. Interkinesis was rarely distinct, but in *C. laxiflora* he observed the haploid number of pairs. He concluded that the first division is equational, and also found some evidence that the second was reductional. His photomicrographs show very small chromosomes, sure interpretations seem difficult. The aspect of first metaphase and interkinesis in *Eleocharis* no doubt shows similarities, but I am not completely convinced of equational division. In *Luzula purpurea* equational division is much more clear: ring-bivalents are transverse to the spindle, the interkinesis nuclei may contain half-bivalents with relic halves of interstitial chiasmata or a ring-of-four may be observed in each interkinesis nucleus. Probably the investigation of a plant with frequent multivalent formation or with heteromorphic bivalents could decide the question of pre- or post-reduction in *Eleocharis*. The hetero-bivalent in *Scirpus lacustris* var. *pictus* is of no use here since it could show reductional as well as equational division of the unpaired segment at first anaphase (TANAKA, *l. c.*). Only equational division was directly observed, however, the occurrence of reductional division was infered from II-metaphase plates lacking the large segment.

The behaviour of the chromosomes in the x-rayed spikelets supports the idea of a diffuse centromere or perhaps rather of polycentric chromosomes: fragments in most cases are in the equatorial plate; later they go to the poles. The effect of x-rays on the divisions was different. Second pollen mitosis was unchanged after 1000 *r*. First pollen mitosis showed supernumerary chromosomes — no doubt due to breakage —, very small fragments, lagging and bridge formation at anaphase. Disturbed spindle mechanism was very frequent in this division. First meiotic division showed supernumerary elements in the form of univalents or fragments, different orientation of bivalents in the plate, structural changes, disturbed anaphase. Second meiotic division could show a larger number of small fragments and frequent lagging and bridge formation at anaphase. The supernumerary elements in pollen mitosis cannot be connected with irregular meiosis in the x-rayed spikelets; 48 hours are too short a time, although it is a fact that in *Cyperaceae* as well as in *Juncaceae* (I have investigated *Juncus effusus*) the first pollen mitosis is earlier than in other Angiosperms. This is also clear from the statement of WULFF (1939) that in *Juncaceae* and in *Cyperaceae* growth of the pollen grain and forming of its exine occur after the pollen mitosis, not before as in other Angiosperms.

Some peculiarities observed after x-raying have also been described

in untreated material. In a single pollen mother cell HICKS observed 9 instead of 8 chromosomes. No doubt the chromosomes present formed 7 II+2 I and there is no reason to ascribe also the chromosome number 9 to *E. palustris*, as has been done in several chromosome lists. As spontaneous variation here was observed 20 (18 II+2 I) chromosomes sometimes and in one plant a supernumerary rather large fragment. Concerning variation in pollen mitosis a statement of WALTERS may be cited, »Intraindividual variation DOXEY found in *E. uniglumis*, a western Kent plant showing at pollen mitosis 19—24 chromosomes while in other plants variation to a lesser extent was found». The actual number of this species is 23 and in this case irregular meiosis may be the cause of the described variation. Meiotic irregularities have been described in different *Carex* hybrids and also in some *Scirpus* forms. As a rule, the second division is more disturbed, lagging and bridge formation being frequent. The irregularities have been ascribed to structural differences and to the inability of a chromosome to divide twice during meiosis; in *Cyperaceae* univalents regularly divide at first anaphase, chromatids later meeting difficulties at the second division.

The frequent lagging after x-raying is no doubt often due to structural changes. The longitudinal position of the lagging chromosome between the poles is an evidence. ÖSTERGREN (1949) assumed that chromosomes with a diffuse centromere have kinetic properties on one side in the form of a kinetic stripe or perhaps a series of chromomeres with kinetic properties. The reunion of fragments after breakage may result in different parts of a chromosome having the kinetic stripe turned in opposite directions. Such a chromosome is pulled to opposite poles and lags in the spindle, sometimes it is not included in the telophase nucleus. Fragments are in most cases in the metaphase plate, this indicates centric properties. Acentric fragments no doubt also occur but comparatively rarely fragments are observed outside the spindle or the nuclei. At late anaphase small centric fragments seem to pass undivided to the pole.

SUMMARY.

The appearance and mitotic behaviour of the chromosomes of *Eleocharis palustris* subsp. *vulgaris* indicate that they have a diffuse centromere or are polycentric. Investigation of meiosis and pollen mitosis in x-rayed spikelets supports this view. It was, however, not possible to show post-reduction with reasonable certainty.

LITERATURE CITED.

1. BROWN, S. W. 1950. Spurious secondary association and asymmetric spindles in a *Luzula*. — Cytologia 15.
2. — 1951. Abstract. — Genetics 39.
3. CASTRO, D. DE, CAMARA, A. and MALHEIROS, N. 1949. X-rays in the centromere problem of *Luzula purpurea* LINK. — Genética Ibérica 2.
4. DOXEY, D. 1938. The cytology of *Eleocharis palustris* and related species in Britain. — Thesis for M. Sc. degree, Manchester (unpublished; cited after WALTERS).
5. GEITLER, L. 1930. Über die Kernteilung von *Spirogyra*. — Arch. Protistenk. 71.
6. HEILBORN, O. 1924. Chromosome numbers and dimensions, species formation and phylogeny in the genus *Carex*. — Hereditas V.
7. — 1939. Chromosome studies in *Cyperaceae*. III—IV. — Hereditas XXV.
8. HELENIUS, O. 1952. The mode of bivalent orientation in *Hemiptera*. — Hereditas XXXVIII.
9. HICKS, G. C. 1928. Chromosome studies in the *Cyperaceae*, with special reference to *Scirpus*. — Bot. Gazette 86.
10. — 1929. Cytological studies in *Cyperus*, *Eleocharis*, *Dulichium*, and *Eriophorum*. — Bot. Gazette 88.
11. HUGHES-SCHRADER, S. and RIS, H. 1942. The diffuse spindle attachment of Coccids, verified by the mitotic behaviour of induced chromosome fragments. — Journ. of exp. Zool. 87.
12. HÅKANSSON, A. 1928. Die Chromosomen einiger *Scirpoideen*. — Hereditas X.
13. — 1929. Über verschiedene Chromosomenzahlen in *Scirpus palustris* L. — Hereditas XIII.
14. KING, G. C. 1953. »Diffuse» centromere and other cytological observations on two Desmids. — Nature 171.
15. KOLLER, P. C. 1943. The effects of radiation on pollen grain development, differentiation and germination. — Proc. Roy. Soc. Edinburgh, B, vol. LXI.
16. LA COUR, L. F. 1953. The *Luzula* system analysed by x-rays. — Suppl. to Heredity 6.
17. LEWITSKY, G. A. 1940. Two karyotypes of some pairs of related forms of plants. — Journ. botanique de l'URSS 25.
18. LIMA-DE-FARIA, A. 1949. Genetics, origin and evolution of kinetochores. — Hereditas XXXV.
19. MALHEIROS, N. CASTRO, D. DE and CAMARA, A. 1947. Cromosomas sem centrómero lócalizado. O caso da *Luzula purpurea* LINK. — Agronomia Lusitana 9.
20. MALHEIROS-GARDÉ, N. and GARDÉ, A. 1951. Agmatoploidia no genero *Luzula* DC. — Genética Ibérica 3.
21. MICHALSKA, A. 1953. Cytological investigations on *Luzula*. — Acta soc. botan. polon. XXII.
22. NORDENSKIÖLD, H. 1951. Cyto-taxonomical studies in the genus *Luzula*. — Hereditas XXXVII.
23. PFEIFFER, H. 1940. Zytologische Studien an der Gattung *Scirpus*. — (Cited after TISCHLER.)
24. PIECH, K. 1924. Über die Teilung des primären Pollenkerns und die Entstehung

der Spermazellen bei *Scirpus palustris* L. — Bull. acad. polon. de sci., ser. B, sc. nat.

25. SCHRADER, F. 1935. Notes on the mitotic behaviour of long chromosomes. — Cytologia 6.
26. SUOMALAINEN, E. 1953. The kinetochore and the bivalent structure in the *Lepidoptera*. — Hereditas XXXIX (Festskrift för ARNE MÜNTZING 2.3.1953).
27. TANAKA, N. 1939. Chromosome studies in *Cyperaceae*. III. The maturation divisions in *Scirpus lacustris* L., with special reference to heteromorphic pairing. — Cytologia 9.
28. — 1940. Ditto. VIII. Meiosis in diploid and tetraploid forms of *Carex sidérosticta* HANCE. — Cytologia 11.
29. — 1950. Chromosome studies in the genus *Carex*, with special reference to aneuploidy and polyploidy. — Cytologia 15.
30. TISCHLER, G. 1950. Chromosomenzahlen der Gefässpflanzen von Mitteleuropa. — Groningen.
31. WAHL, H. A. 1940. Chromosome numbers and meiosis in the genus *Carex*. — Amer. Journ. Botany 27.
32. WALTERS, S. M. 1949. *Eleocharis* R. BR. (Biological flora of the British Isles). — Journ. of Ecology 37.
33. WULFF, H. D. 1939. Die Pollenentwicklung der Juncaceen nebst einer Auswertung der embryologischen Befunde hinsichtlich der Verwandtschaft der Juncaceen und Cyperaceen. — Jahrb. wiss. Botanik 87.
34. ÖSTERGREN, G. 1949. *Luzula* and the mechanism of chromosome movement. — Hereditas XXXV.

CONTENTS.

Introduction 325
Mitosis in roots 327
First pollen mitosis 327
Second pollen mitosis 332
Meiosis 332
Conclusions 337
Summary 343
Literature cited 344

ON ACCESSORY CHROMOSOMES IN FESTUCA PRATENSIS

I. CYTOLOGICAL INVESTIGATIONS

BY *NILS OLOF BOSEMARK*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

I. INTRODUCTION.

NORMALLY, *Festuca pratensis* HUDS. has seven pairs of chromosomes. RANCKEN (1934), in a study of the cytology of some grasses, reported the occurrence of what he called fragment chromosomes in some plants of *Festuca pratensis*, among other species. These fragment chromosomes seem to behave similarly to the B-chromosomes in maize, *Sorghum* and *Anthoxanthum* and the standard fragment in rye.

The purpose of the present investigation has been to compare the behaviour and effect of these chromosome elements in *Festuca pratensis* with those found in the materials mentioned above, and others. In this paper, which is the first in a series of publications, I will call these chromosomes *accessory chromosomes* following the terminology suggested by HÅKANSSON (1945) and MÜNTZING (1945). In a preliminary note (BOSEMARK, 1950) I have presented some of the earlier data on the accessory chromosomes in *Festuca pratensis*. For a review of the present knowledge on accessory chromosomes in plants, see MÜNTZING (1949, 1954) and TISCHLER (1953).

II. MATERIALS AND METHODS.

Festuca pratensis is a typical cross-fertilizer with high variability in minor morphological characters, vitality and fertility, characteristic of such plants. The material for the present investigation consists mainly of plants collected from wild populations in Sweden or is derived from such plants. The results of the collections with respect to frequency and geographical distribution of the accessory chromosomes will be considered in detail in another paper. Some data from the material collected in 1948—49, have been given already in the previous report (BOSEMARK, 1950).

For chromosome counts root-tips were fixed in chrome-acetic

formalin (MÜNTZING's modification of NAVASHIN's fluid; MÜNTZING, 1933) and sectioned at 14 μ. The study of meiosis has been made mainly in squashes after fixing in acetic-alcohol (1 : 3), but also in sections of 20 μ fixed in chrome-acetic formalin after prefixing in acetic-alcohol. All sections were stained in crystal violet, squashes in aceto-carmine or Feulgen. The first pollen mitosis has been studied in aceto-carmine squashes after fixing in acetic-alcohol. Further details on the technique used will appear in the text.

III. MITOSIS.

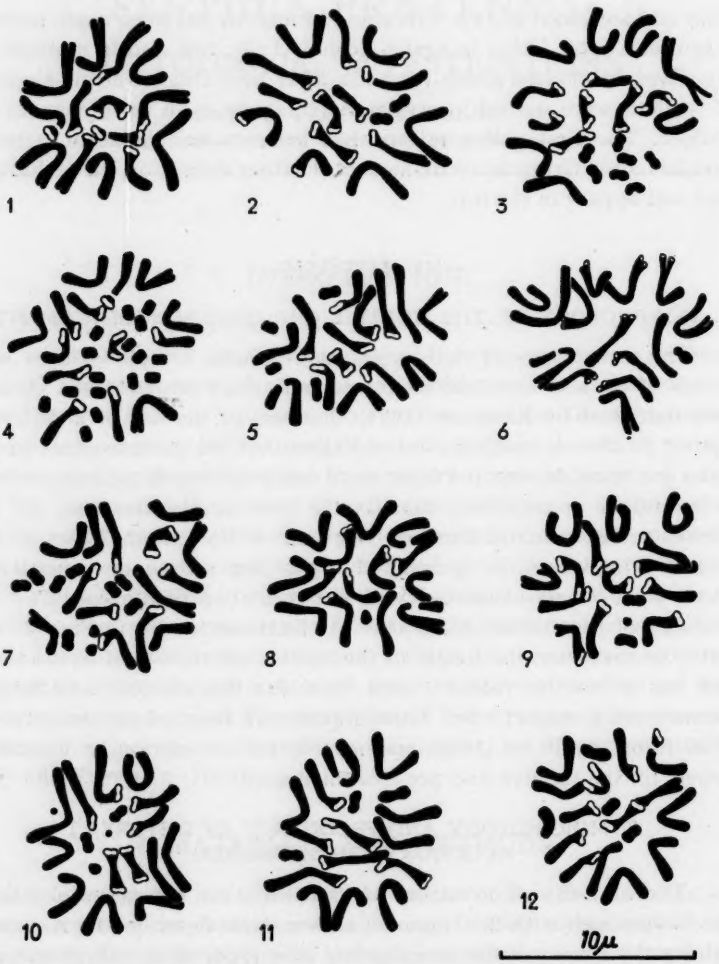
1. MORPHOLOGY OF THE NORMAL CHROMOSOME COMPLEMENT.

The morphology of the normal chromosome complement, as well as that of the accessory chromosomes, in *Festuca pratensis* has already been described by RANCKEN (1934). Because of the use of a different type of fixative (a modification of FLEMMING) the chromosomes in his slides are more slender and have more easily detectable primary as well as secondary constrictions than is the case in my fixations. In the chrome-acetic formalin fixations one pair of the chromosomes in the normal complement in general shows a big secondary constriction which is obviously concerned with the formation of nucleoli. The appearance of the chromosomes after my fixations is seen in Figs. 1—12. RANCKEN measured the length of the normal chromosomes in his slides and has given the values 4 and 6,5 μ for the shortest and longest chromosomes, respectively. These figures are in good agreement with those found by PETO (1933) and myself, but are obviously dependent largely on the fixative and pre-treatment used.

2. MORPHOLOGY AND FREQUENCY OF DIFFERENT ACCESSORY CHROMOSOMES.

The majority of accessory chromosomes in my material corresponds very well with the fragment chromosome described by RANCKEN. During the course of the investigation new types of accessory chromosomes, however, have been found in wild material as well as in progenies after crossing plants with accessory chromosomes of the standard type. For that reason it has been necessary to name the different types in the following way:

(a) *The standard type of accessory chromosomes* (*st. acc.*). — This is the dominating type. Its length is about $^1/_4$ of the mean for the normal chromosomes, it has a median centromere and is of the same thickness



Figs. 1—12. Somatic metaphase plates in root-tips from plants with a varying number of accessory chromosomes of different types. — Fig. 1, $2n=14$. — Fig. 2, $2n=14+2$ st. acc. — Fig. 3, $2n=14+7$ st. acc. — Fig. 4, $2n=14+16$ st. acc. — Fig. 5, $2n=14+2$ st. acc.$+2$ s. acc. — Fig. 6, $2n=14+1$ s. acc. — Fig. 7, $2n=14+8$ st. acc.$+2$ s. acc. — Fig. 8, $2n=14+1$ s. acc. — Fig. 9, $2n=14+2$ st. acc.$+1$ v. s. acc. — Fig. 10, $2n=14+2$ st. acc.$+1$ s. acc.$+2$ v. s. acc. — Fig. 11, $2n=14+2$ st. acc.$+1$ l. acc. — Fig. 12, $2n=14+2$ st. acc.$+2$ l. acc.

as a normal chromosome (Figs. 1—4). Nearly all data in the present paper are derived from plants with accessory chromosomes of this type only. Thus, accessory chromosome in the text and tables refers to this type, unless otherwise stated.

(b) *Small accessory chromosomes* (*s. acc.*). — This group consists of all accessory chromosomes of smaller size, not smaller than half a standard accessory chromosome. Thus this group, as also the following, is not homogeneous, and a number of different types can be distinguished (Figs. 5—8).

(c) *Very small accessory chromosomes* (*v. s. acc.*). — All accessory chromosomes smaller than half the standard type. The smallest ones in this category appear as small dots in root-tip metaphases (Figs. 9—10).

(d) *Large accessory chromosomes* (*l. acc.*). — In a few plants chromosomes of about double the size of a standard accessory chromosome have been found. They are not yet studied in detail, but at least some seem to behave as accessory chromosomes, others undoubtedly are real fragments from the normal complement. Until better understood the first category will be described as large accessory chromosomes (Figs. 11—12).

Most of the plants with deviating accessory chromosomes have been met with among progeny plants after crosses or open pollination of plants with higher numbers of accessory chromosomes of the standard type. This and cytological observations make it highly probable that the smaller types have originated through fragmentation of the standard type. Such accessory chromosomes of a smaller size, derived from a standard type, have already been described in maize (RANDOLPH, 1928, 1941; LONGLEY, 1938; and DARLINGTON and UPCOTT, 1941) and rye (MÜNTZING, 1944, 1948 b; and MÜNTZING and LIMA-DE-FARIA, 1952). In maize, as in my material, there is a whole series of accessory chromosomes, which have been called B-, C-, D-, E- and F-chromosomes (B=standard). Cytological studies have demonstrated that probably all of these smaller types have originated from the B-chromosome (LONGLEY, 1938; RANDOLPH, 1941). MÜNTZING (1944) has described how misdivision of the standard fragment in rye gives rise to two new accessory chromosomes of iso-chromosome type, one larger and one smaller than the standard fragment. MÜNTZING (1948 b) also found another smaller type of accessory chromosome in rye, thought to have originated through a deletion in one arm of the standard fragment. A pachytene analysis (MÜNTZING and LIMA-DE-FARIA, 1952), however, revealed it to be due

to a terminal deficiency. The origin of the large accessory chromosome in *Festuca pratensis* is still obscure. As it is of about double the size of the standard accessory chromosome and has a more or less median centromere, it is not possible to explain its origin through misdivision of the standard type similar to that in rye, unless the centromere attained a more terminal position by an inversion before such a misdivision occurred. Further studies of the cytology and inheritance of the different types of accessory chromosomes are in progress.

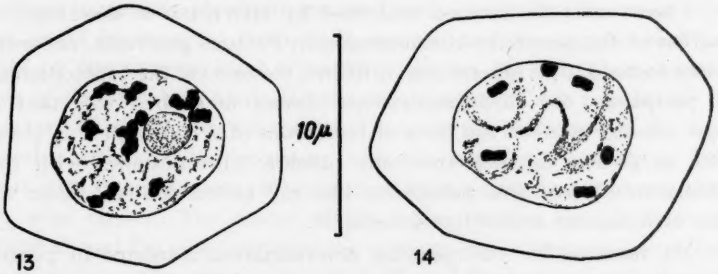
3. HETEROCHROMATIC NATURE OF THE ACCESSORY CHROMOSOMES.

The majority of accessory chromosomes in plant as well as animal material is known to be more or less heterochromatic. This is also the case with the accessory chromosomes in *Festuca pratensis*. The heterochromatic nature of the accessories in my material has been studied in root-tips as well as pollen mother cells and pollen grains. Here I will consider only the situation in root-tips, the other results will be discussed later. It should be pointed out that the terms heterochromatin and heterochromatic in this paper, in accordance with the definition by HEITZ (1932), are used only as morphological concepts. The terms positive and negative heteropycnosis were introduced by WHITE in 1935; his definition of heterochromatin, based on these terms (WHITE, 1945), is not in disagreement with HEITZ's original definition, and will also be used. The normal chromosome complement of *Festuca pratensis*, judging from resting nuclei and early prophase stages in root-tips without accessory chromosomes, does not contain any larger heterochromatic segments. Resting nuclei in plants without accessory chromosomes have a varying number of small overstained bodies. In plants with accessory chromosomes, however, depending on their number, one or more large overstained bodies are present in the resting nuclei (Fig. 13). These chromocentres or prochromosomes can be of different size and more or less well defined, but are generally so marked that judging from resting nuclei and early prophase stages, one can say with a fairly high accuracy whether a plant has accessory chromosomes or not (Fig. 14). In plants with high numbers of accessory chromosomes the individual chromocentres are often associated with each other forming larger units.

At metaphase the stainability of the accessory chromosomes with crystal violet, orcein or Feulgen is quite as good as that of the normal chromosomes, and I have not been able to find any negative heteropycnosis at this stage after moderate cold treatment, as described for

the accessory chromosomes in *Anthoxanthum aristatum* (ÖSTERGREN, 1947). This may, however, depend on the difficulty of measuring small differences in thickness in the relatively small accessory chromosomes in *Festuca pratensis*.

Behaviour similar to that now described for the accessory chromosomes in *Festuca* has been found in *Sorghum* (DARLINGTON and THOMAS, 1941), maize (MORGAN JR., 1943) and *Anthoxanthum* (ÖSTERGREN, 1947). MORGAN made a careful investigation of the relationship between number of knobs and number of chromocentres, as well as between B-chromosomes and chromocentres in maize, and found a significant positive correlation between number of chromocentres and number of



Figs. 13—14. Positive heteropycnosis in root-tips from plants with accessory chromosomes. — Fig. 13, Resting nucleus in a plant with $2n=14+14$ st. acc. — Fig. 14, Prophase in a plant with $2n=14+6$ st. acc.

knobs and B-chromosomes, respectively, though as, in *Anthoxanthum* and in my material, association of chromocentres in general led to a lower figure for these than for number of knobs and B-chromosomes. ÖSTERGREN points out that the degree of heteropycnosis varies within the same root in such a way that in the higher regions of the root the heterochromatin is more easily seen than in vigorously growing meristematic cells. This also holds true to some extent for my material, but still more striking differences can sometimes be seen owing to the physiological condition of the roots at the time of fixation.

4. THE POSITION OF THE ACCESSORY CHROMOSOMES IN SOMATIC METAPHASE PLATES.

MÜNTZING (1948 a) has shown that the different types of accessory chromosomes in rye have a more or less central position in root-tip metaphase plates depending on their size in such a way that the smallest

have the most central position. All types of accessory chromosomes in his material, except the large iso-fragment, which is of about the same size as the normal chromosomes, have a more central position than these. This is in agreement with the general experience that in somatic metaphase plates with chromosomes of different size, smaller chromosomes tend to have a more central position than larger ones.

In maize (DARLINGTON and UPCOTT, 1941) the B-chromosomes, though smaller than the normal chromosomes, have a very strong tendency to be situated on the periphery of the somatic plates. This is thought to be due to the B-chromosomes having a weaker centromere. MÜNTZING concludes from his data in rye that the accessory chromosomes in this material have centromeres of a normal strength.

I have used the method described by MÜNTZING to determine the position of the accessory chromosomes in *Festuca pratensis*. According to this method a peripheral line is drawn connecting the centromeres in the periphery; the chromosomes are chosen in such a way that the angle formed between the lines at both sides of a centromere is always 180° or greater as seen from the outside. Chromosomes with their centromeres inside this peripheral line are called central, those with their centromeres on the line peripheral.

My material for this position determination consisted of progeny plants from two crosses and one single plant, the latter with a fairly high number of accessory chromosomes. The result is seen in Table 1 (P=peripheral chromosomes, C=central chromosomes and % C=per cent central chromosomes).

From the statistical analysis it is evident that the accessory chromosomes in *Festuca pratensis*, as in rye, have a more central position in somatic metaphase plates than the normal chromosomes. Judging from this the accessory chromosomes in *Festuca pratensis* do not have defective

TABLE 1. *The position of the accessory chromosomes in somatic metaphase plates.*

Number of acc. chr.	Material	Normal chr.			Acc. chr.		
		P	C	% C	P	C	% C
14+2 st. acc.	K1/50	1141	805	41,4	118	160	57,6
14+2 » »	K65/50	501	297	37,2	50	64	56,1
14+4 » »	K1/50	328	218	39,9	59	97	62,2
14+7 » »	pl. 212	171	109	38,9	54	86	61,4
14+2—7 st. acc.	Total	2141	1429	40,0	281	407	59,2

χ^2 (total) = 86,06 $P < 0{,}001$

TABLE 2. *Analysis of expected somatic pairing between accessory chromosomes in plants with 2n=14+2 st. acc.*

Material	Angles									
	0°	20°	40°	60°	80°	100°	120°	140°	160°	180°
K135/48	2	2	7	3	3	2	4	2	4	
K1/50	4	3	5	4	1	3	1	1	1	
K65/50	2	2	1	2	1	—	2	—	3	
Total	8	7	13	9	5	5	7	3	8	

centromeres. The different progenies used in my investigation seem to behave in quite the same way.

In his paper of 1934 RANCKEN points out that the accessory chromosomes in somatic plates are grouped two or more together more often than could be expected from mere chance, and he suggests that this might be due to some kind of somatic pairing. To test this hypothesis some of the drawings made for the investigation of the position of the accessory chromosomes were used. To avoid the influence of the more central position of the accessory chromosomes, only drawings from plants with two accessory chromosomes, both situated on the peripheral line, were chosen. The centre of the figure formed by this line was estimated, and the angle between the centromeres of the accessory chromosomes from the centre measured. If any form of somatic pairing exists, one should expect small angles to dominate, if else all angles between 0° and 180° should be equally represented (Table 2).

As seen from the table, there is a low excess of angles smaller than 90° (0°—90°=39,5 cases; 90°—180°=25,5 cases), but a χ^2-test reveals that this distribution does not deviate significantly from a 32,5 : 32,5 distribution ($\chi^2=3{,}015$; $0{,}10 > p > 0{,}05$) which is the expected one without somatic pairing. In this material there is thus no evidence for somatic pairing. The fact that one easily gets this impression, especially in plants with high numbers of accessory chromosomes, is probably the result of the more central position of these chromosomes; this will lead to a reduced distance between them. The frequent association of accessories into chromocentres in the mitotic interphase may also influence their position at metaphase.

5. CONSTANCY OF THE ACCESSORY CHROMOSOMES WITHIN PLANTS.

Among plants with accessory chromosomes there are two species where these chromosomes are present only in the germ-line and are

eliminated in the root-tips and other somatic tissues: *Sorghum* (DARLINGTON and THOMAS, 1941) and a sexual strain of *Poa alpina* (MÜNTZING, 1946 b, 1948 c). In some other materials there is a certain variation between root-tips, or even within the same root-tip, from the same individual. The best known cases of this type are *Zea mays* (GROSSMAN, 1937; *cf.* TISCHLER, 1953; and DARLINGTON and UPCOTT, 1941), *Godetia viminea* (HÅKANSSON, 1949) and *Godetia nutans* × *Whitneyi* with acc. chr. (HÅKANSSON, 1950). In contrast to this it has been reported for rye (MÜNTZING, 1948 a) and *Anthoxanthum* (ÖSTERGREN, 1947) that the accessory chromosomes are constant within one individual with few exceptions. The accessory chromosomes in *Festuca pratensis* in this respect behave like those in rye and *Anthoxanthum*. Only in a few slides out of several thousands has it been possible to establish with any certainty a variation of ±1 accessory chromosome. Also the smaller types of accessory chromosomes seem to have a fairly high degree of constancy in the root-tips.

IV. MEIOSIS.

Meiosis has been studied in detail in altogether 69 plants with a standard accessory chromosome number distribution as listed in Table 3.

1. PLANTS WITHOUT ACCESSORY CHROMOSOMES.

The seventeen plants without accessory chromosomes were all taken at random from material collected in nature or obtained from crosses between such plants. Most of them had a regular meiosis with seven pairs at metaphase I. There is a variation between individuals with respect to chiasma frequency, but most cells have six ring bivalents and one rod bivalent (Fig. 32). Some plants show univalents, non-oriented bivalents and delayed anaphase separation in low frequency. Later stages in meiosis are, in general, regular. This is in conformity with the findings of PETO (1933), RANCKEN (1934) and MYERS and HILL (1947). Besides these seventeen plants about thirty plants from the collections in 1948—49, most of which had no accessory chromosomes, were examined cytologically because of their marked sterility on the

TABLE 3. *Number of plants with respective numbers of accessory chromosomes of the standard type studied in meiosis.*

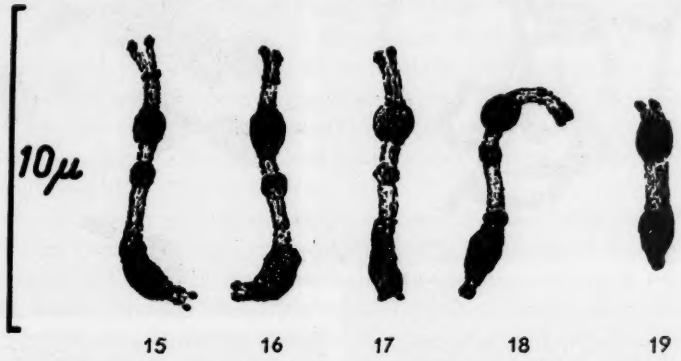
Number of acc. chr.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Number of plants	17	18	17	—	5	—	—	1	1	1	4	2	—	—	1	1	1

male side. Out of these three were translocation heterozygotes, three carried an inversion and one was partially asynaptic. The rest of these plants showed regular meiosis or only minor irregularities.

2. PLANTS WITH ACCESSORY CHROMOSOMES.

A. GENERAL BEHAVIOUR OF ACCESSORY CHROMOSOMES IN MEIOSIS.

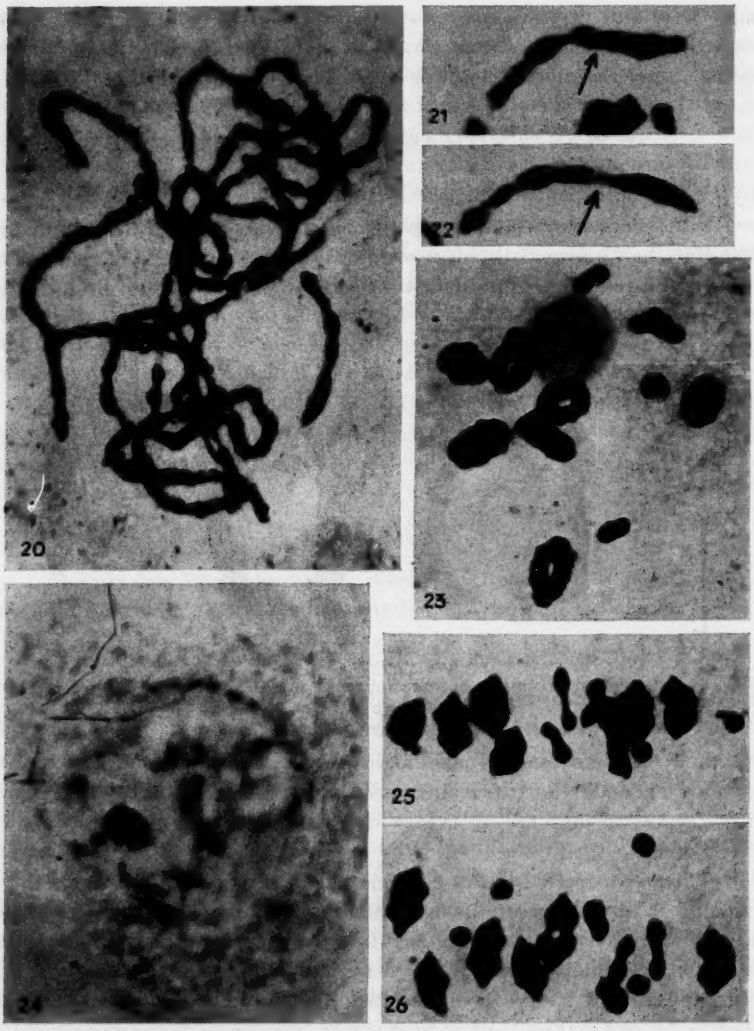
When accessory chromosomes were first encountered it was thought that they had arisen through a *simple* fragmentation of a chromosome from the normal complement. As a result of this they were often called



Figs. 15—19. Paired accessory chromosomes of the standard type in meiotic prophase. — Figs. 15—18, pachytene. — Fig. 19, diplotene.

fragment chromosomes. In zoological materials the similarity in behaviour between the extra chromosomes and sex chromosomes sometimes suggests that the accessory chromosomes have originated from the latter.

Through careful studies of meiotic pairing and chromosome morphology it has, however, been possible, in many botanical as well as zoological materials, to prove that the accessory chromosomes have not arisen immediately through a *simple* fragmentation of the ordinary chromosomes. It is very probable that they initially originated through such a fragmentation, but subsequently they must have undergone a number of structural re-arrangements including deletion of euchromatic and duplication of heterochromatic segments (WHITE, 1945). This opinion is based on the lack of pairing between accessory chromosomes and the normal ones and on the results of careful comparisons of their morphology at pachytene. Pachytene analyses of accessory chromosomes



Figs. 20—22. Paired standard accessory chromosomes at pachytene. — Fig. 20, Pachytene in a plant with $2n=14+2$ st. acc. (1650×). — Figs. 21—22, Paired st. acc., arrow indicates probable position of the centromere (2500×). — Fig. 23, Diakinesis in a plant with $2n=14+10$ st. acc. (1550×). — Fig. 24, Positive heteropycnosis of acc. chr. at diplotene-diakinesis in meiosis, $2n=14+2$ st. acc. (3000×). — Figs. 25—26, Metaphase I in plants with $2n=14+2$ st. acc. (1550×).

have been made in maize (McCLINTOCK, 1933) and in rye (LIMA-DE-FARIA, 1948, 1952; MÜNTZING and LIMA-DE-FARIA, 1949). In many plant materials the accessory chromosomes are heterochromatic at meiotic prophase. This is the case in maize (McCLINTOCK, 1933), *Sorghum* (DARLINGTON and THOMAS, 1941), *Poa alpina* (MÜNTZING, 1948) and to some extent also in rye (LIMA-DE-FARIA, 1952). The accessory chromosomes of *Festuca pratensis* fall into this group. In sectioned material stained with crystal violet the prophase accessory chromosomes generally retain the stain better than do the normal chromosomes (Fig. 24); in aceto-carmine squashes this has been found to be due to the presence of large heterochromatic segments (Figs. 15—19, 20—22). Although I have not made a complete analysis of the normal chromosome complement at the pachytene stage, I consider it unlikely that any of the normal chromosomes have heterochromatic segments as large as those of the accessory chromosomes. At diplotene and diakinesis the accessories often have a smoother outline and look more compact than do the normal chromosomes (Fig. 23).

The accessory chromosomes in *Festuca pratensis* resemble other accessories in not pairing with chromosomes of the normal complement. Sometimes one can see a connection between accessory and normal chromosomes [this has been reported in the present species by RANCKEN (1934)], but this occasionally happens also between normal chromosomes and is not due to real pairing; the slightly higher frequency for the accessory chromosomes is probably caused by their more sticky properties. Similar observations have been made on the accessory chromosomes in *Anthoxanthum* (ÖSTERGREN, 1947) and *Poa alpina* (MÜNTZING, 1948 c), among other materials.

Some remarks on accessory chromosome origin have already been made in this section. Before going on to the next topic, I should like to point out that nothing definite can as yet be said about the origin of the accessories in *Festuca pratensis*, except that it is very unlikely that they arise through a simple fragmentation of a normal chromosome. Besides the pairing behaviour and the morphology, some other facts strengthen this conclusion. All the accessory chromosomes of standard size, which are of overwhelming majority in my material, have, as far as one can see, the same morphology at mitosis whether they are studied in plants from Sweden, Denmark, Holland, England, Germany or Italy. This speaks in favour of the idea, brought out by ÖSTERGREN (1947) among others, that the accessory chromosomes are of very old, common origin. If, on the other hand, there is a new-production going on, this must be

highly specialized. In rye (MÜNTZING, 1950) accessory chromosomes from Turkey have a pachytene structure only slightly different from that found in Swedish rye.

B. MEIOSIS IN PLANTS WITH ONE STANDARD ACCESSORY CHROMOSOME.

At pachytene in these plants the two arms of the accessory chromosome often seem to be paired, just as the univalent B-chromosome in maize (MCCLINTOCK, 1933). That this does not mean that the accessory chromosome is of the iso-chromosome type, but the result of its heterochromatic nature, is seen from the studies of paired accessories at pachytene (Figs. 15—18, 20—22). At diplotene, and especially diakinesis, the univalent accessory chromosome appears as a more or less irregular dot.

The position of the univalent accessory chromosome at metaphase I and anaphase I has been carefully studied in a fairly large number of plants (Table 4).

As seen from the table, the univalent has a striking ability to orient itself on the plate at metaphase I. At present, material is not available for a strict comparison between accessories and univalents from the normal complement in this respect. Counts in a partially asynaptic plant without accessory chromosomes gave a significantly lower figure for these univalents with respect to metaphase orientation, but, as pointed out by ÖSTERGREN and VIGFUSSON (1953), the number of univalents present in a cell seems to affect their position, and thus it should

TABLE 4. *Position of the univalent accessory chromosome at metaphase I and mid-anaphase I in plants with 2n=14+1 st. acc.*

	Metaphase I			Mid-anaphase I		
Plant No.	% outside the plate	% on the plate	Number of cells	% at one of the poles	% between poles	Number of cells
84	54	46	247	40	60	30
94	40	60	78	38	62	13
102	56	44	398	25	75	110
129	37	63	271	32	68	31
131	35	65	479	32	68	207
152	34	66	169	17	83	18
163	29	71	329	22	78	104
164	35	65	215	—	—	—
173	39	61	258	39	61	67
297	26	74	173	22	78	40
366	26	74	65	11	89	46
Total	39	61	2682	28	72	666

be necessary to have trisomic, triploid or asynaptic plants with accessory chromosomes for a strict comparison. As will be pointed out later, generally 60 % of the univalents, irrespective of their total number, orient themselves on the plate. This speaks against such a stipulated effect of increasing number of univalents on their orientation, at least in so far as accessory chromosomes in *Festuca pratensis* are concerned. It is also obvious from the table that there is a marked variation between plants with respect to metaphase orientation. The figures for accessory chromosomes between poles at mid-anaphase are a little higher than corresponding figures at metaphase. This indicates that in some of the metaphase plates, the accessory chromosome in fact had not completed its orientation. Though care was taken to score only full metaphase, pro-metaphase stages may, in spite of the large number of cells scored, be the cause of the large discrepancy between the figures for metaphase I and anaphase I in plants 84 and 102.

Univalent accessory chromosomes left between the poles regularly divide at late anaphase. The elimination of accessories at this stage is, judged from the frequency of micronuclei at telophase I, very low. Dividing univalent accessories at late anaphase I, however, show a more or less marked negative heteropycnosis compared to the normal chromosomes. Thus it might be possible that some divided accessories are resorbed in the plasm before reaching the telophase nucleus, without forming a micronucleus.

At metaphase II the accessory chromosomes, whether divided or not, frequently orient themselves on the plate. This results in a fairly high frequency of lagging accessories at second anaphase. That most of these have divided at first anaphase is obvious from the fact that very often both daughter cells show laggards. As in the earlier stages there is a variation between individuals with respect to frequency of lagging at this stage (Table 5).

TABLE 5. *Lagging accessory chromosomes at anaphase II in plants with 2n=14+1 st. acc.*

Plant No.	Number of cells without lagging acc. chr.	Number of cells with lagging acc. chr.	Per cent cells with lagging acc. chr.	Total number of cells
102	25	32	56	57
131	56	41	42	97
163	86	38	31	124
239	41	65	61	106

$\chi^2 = 24{,}699$ $P < 0{,}001$

From the high numbers of already divided univalents at anaphase II one should expect a fairly high elimination and frequency of micronuclei in the tetrads, but this is not the case. In hundreds of tetrads from different plants in general only a low per cent show micronuclei. The laggards thus have a good ability to move to one of the poles and get included in the nucleus. This has been verified by studying various stages at anaphase II; the frequency of laggards observed steadily decreases towards telophase II. ÖSTERGREN (1947) similarly found that univalents lagging at anaphase I, although they did not divide, in general were included in one of the nuclei.

The univalent accessory chromosome in *Festuca pratensis* combines in it properties from accessory chromosomes in several other materials, but also has some characteristics of its own. It has a variable but generally strong tendency to orient itself on the metaphase plate in metaphase I. Like the accessories in *Zea mays*, *Secale cereale* and *Phleum phleoides* (BÖCHER, 1950), but in contradiction to *Anthoxanthum aristatum* and *Poa alpina*, lagging univalents divide at anaphase I. ÖSTERGREN suggested that lack of division at first anaphase is one reason for a scarcity of micronuclei at the tetrad stage in *Anthoxanthum*. The present material shows that division of univalent accessories at first anaphase does not necessarily mean a high degree of elimination at later stages. The accessory chromosomes in *Festuca* resemble the F-chromosomes in *Trillium* (SPARROW, POND and SPARROW, 1952) in showing great variability in behaviour between different individuals. There are also the aforementioned indications that the accessories in *Festuca* are negatively heteropycnotic at late anaphase in both divisions.

C. PLANTS WITH TWO STANDARD ACCESSORY CHROMOSOMES.

Where it has been possible to study the accessories in pachytene they have always been paired, forming a bivalent of characteristic shape (Figs. 15—18, 20—22). The centromere is situated somewhere between the two largest central heterochromatic segments. In the general description of the accessory chromosomes at meiosis I have already referred to their differential stainability and heterochromaty. Not enough plants have been analysed at this stage to ascertain whether or not accessories from different populations have always the same pachytene morphology, but the general picture has been the same in more than ten plants of different origin. At diplotene it is generally only possible to see one large heterochromatic segment on each side of the centromere (Fig. 19). Also at this stage the accessories are nearly always paired,

while at diakinesis univalents are a little more common. At metaphase I, finally, there is a rather high variation between plants with respect to pairing, and also to some degree between anthers from the same plant. Counts at diakinesis in ten plants gave a mean value for per cent cells with paired accessory chromosomes of 94,5 with a variation between 84—100, while the figure for fourteen plants at metaphase was 72,0 with a variation from 47—93. The difference between the values at diakinesis and metaphase is probably due to lack of chiasma formation in some of the associations seen in the earlier stages. As the accessory chromosomes are heterochromatic it is possible that many of the metaphase associations also lack chiasmata and merely stick together.

Since univalent accessories left between the poles divide at anaphase I, the amount of pairing at metaphase I affects the number of lagging accessories at anaphase II and also to some extent the number of tetrads with micronuclei. This is seen in Table 6.

TABLE 6. *Meiosis in four plants with 2n=14+2 st. acc.*
(Figures calculated per 100 microsporocytes.)

Plant No.	Paired acc. chr. at M I	Univalent acc. chr. lagging at A I	Acc. chr. lagging at A II	Tetrads with micronuclei
K1/50 : 16	72	22	13	3
K13/50 : 7	69	20	10	6
K13/50 : 30	57	51	25	4
K65/50 : 2	47	47	33	24

The position of univalent accessory chromosomes at metaphase I in these plants has been studied, and here, as in plants with only one accessory chromosome, about 60 per cent are oriented on the plate. In general very few accessories are eliminated at late anaphase I and telophase I, and in the second division most accessories are included in the nuclei. Micronuclei in tetrads seldom appear in higher frequencies than 10 per cent. Tetrad micronuclei caused by accessory chromosomes are, however, often more difficult to detect than those from normal chromosomes, and thus may have been overlooked in some cases.

D. MEIOSIS IN PLANTS WITH HIGHER NUMBERS OF STANDARD ACCESSORY CHROMOSOMES.

In plants with higher numbers of accessory chromosomes the pachytene stage is difficult to study, because the accessories generally

TABLE 7. *Pairing behaviour at diakinesis and metaphase I in plants with 2n=14+4 st. acc.*

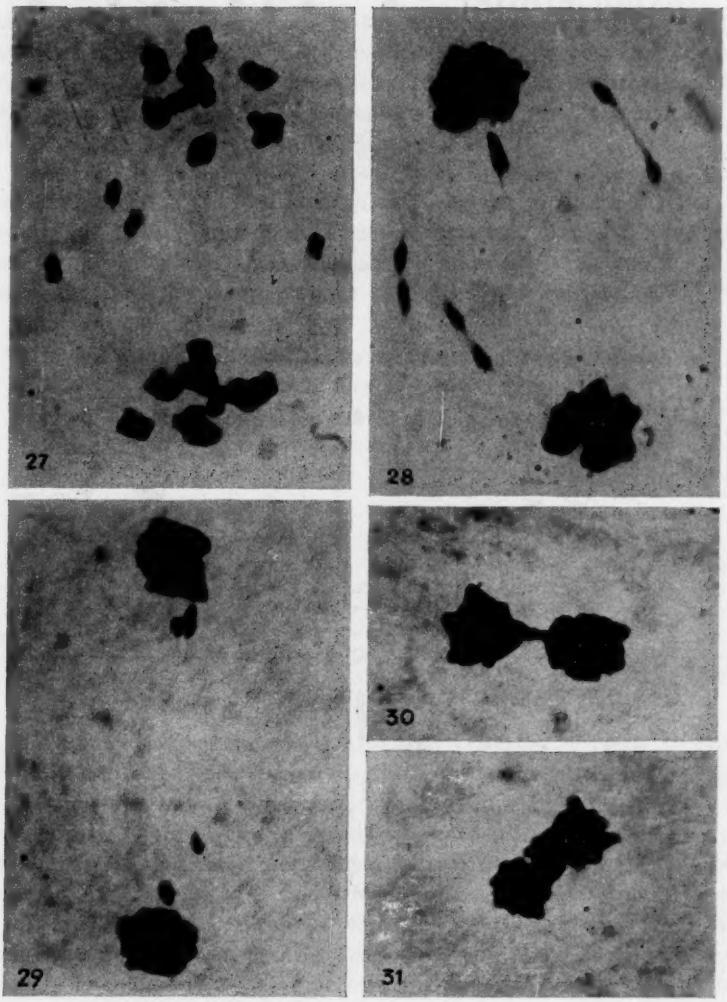
Plant No.	Stage	Number of cells with respective configurations					Total number of cells
		$^1_{IV}$	$^1_{III}+^1_I$	$^2_{II}$	$^1_{II}+^2_I$	^4_I	
K1/50 : 4	Diakinesis	4	2	24	2	—	32
K11/50 : 22	»	9	10	24	7	—	50
K1/50 : 4	Metaphase I	—	—	15	37	31	83
K11/50 : 22	» »	—	—	10	18	7	35

all clump together. It is thus not yet known whether real multivalents are formed at this stage.

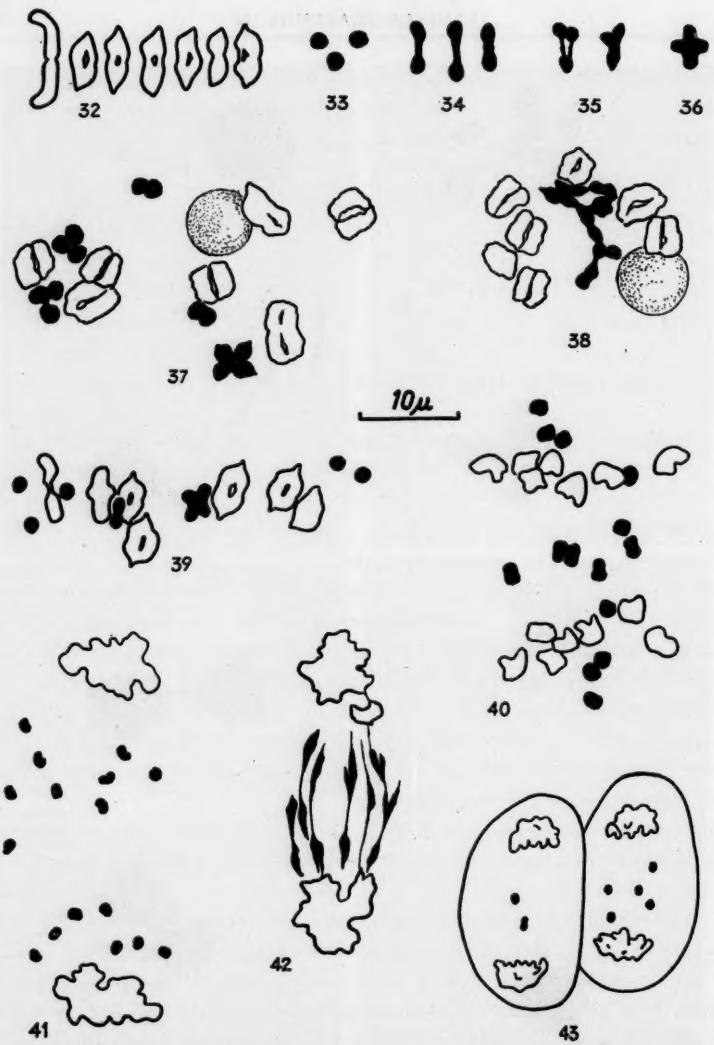
In plants with four accessory chromosomes a comparison between pairing at diakinesis and metaphase I has been made. An example is given in Table 7.

As is seen from the table, higher configurations than bivalents and few univalents occur at diakinesis in these plants, while at metaphase no multivalents but rather many univalents are present. This is in line with what has been said earlier about the sticky properties of the accessory chromosomes and probable lack of chiasma formation in some of the associations. This stickiness in general increases with increasing number of accessories, and in plants with still higher numbers multivalents are found also at metaphase I. The metaphase pairing in plants with four accessory chromosomes is highly variable, and counts in four plants gave the figures 13,5; 40,0; 59,6 and 68,5 for per cent univalent accessories, with a mean value of 45,4. With respect to the other stages the accessory chromosomes behave in the same way as in plants with one and two.

In plants with 7, 8 and 9 accessory chromosomes metaphase I, anaphase I and II and tetrads were analysed. In metaphase I higher configurations than bivalents were seen in the plants with 8 and 9 accessories, but in spite of this, the number of univalents was high, amounting to about 60 per cent of all accessories; of these 60—65 % were oriented on the metaphase plate. At anaphase I in the plant with eight accessory chromosomes univalent accessories had difficulties in dividing and remained undivided until very late anaphase. When they finally started to separate they formed one or more bridges, which broke fairly late when the accessories were not far from the poles (Fig. 42). The same tendency has also been seen in plants with lower numbers of accessory chromosomes, but it is more frequent in plants with many



Figs. 27—31. Meiosis in plants with a fairly high number of accessory chromosomes of the standard type. — Fig. 27, Lagging univalent acc. chr. at anaphase I. — Figs. 28—29, Dividing univalent acc. chr. at late anaphase I and telophase I. — Figs. 30—31, Formation of restitution nuclei after anaphase I due to a high number of univalent acc. chr. unable to divide properly. (1550×).



Figs. 32—43. Meiosis in plants with high numbers of accessory chromosomes of the standard type. — Fig. 32, Bivalents from the normal complement at metaphase I, separately drawn. — Figs. 33—36, Different configurations of acc. chr. at metaphase I; univalents, bivalents, trivalents and a tetravalent. — Fig. 37, Diakinesis in a plant with $2n=14+14$ st. acc. — Fig. 38, Another cell from the same plant, all acc. chr. stick together. — Fig. 39, Metaphase I in a plant with $2n=14+11$ st. acc. — Fig. 40, Anaphase I in a plant with $2n=14+14$ st. acc. — Fig. 41, Late anaphase I in the plant with $2n=14+16$ st. acc. mentioned in the text. Univalent acc. chr. have divided without difficulties. — Fig. 42, Same stage in the plant with $2n=14+15$ st. acc. Dividing acc. chr. have a tendency to form bridges. — Fig. 43, Anaphase II in the plant with $2n=14+16$ st. acc.

accessories; there it also leads to the more severe disturbances to be described later on. Although the number of lagging accessories at early anaphase II in these plants was rather high, most of them reached the poles in late anaphase II and telophase II and, thus, the number of micronuclei in the tetrads was quite low.

Four plants with $2n=14+10$ acc. were carefully analysed in most stages, and will be treated here in more detail. At diakinesis the accessories in these plants often formed associations of 5—6—7 chromosomes, and sometimes all the accessories were united to one irregular lump (Fig. 38). The configurations at metaphase I are seen in Table 8.

TABLE 8. *Configurations of accessory chromosomes at metaphase I in plants with $2n=14+10$ st. acc.*

Plant No.	Number of cells with respective configurations															Total number of cells
	$_{1V}+_{2II}+_{1I}$	$_{1IV}+_{2II}+_{2I}$	$_{1IV}+_{6I}$	$_{2III}+_{2II}$	$_{2III}+_{1II}+_{2I}$	$_{2III}+_{4I}$	$_{1III}+_{3II}+_{1I}$	$_{1III}+_{2II}+_{3I}$	$_{1III}+_{1II}+_{5I}$	$_{5II}$	$_{4II}+_{2I}$	$_{3II}+_{4I}$	$_{2II}+_{6I}$	$_{1II}+_{8I}$	$_{10I}$	
K11/48/8 : 21	—	—	—	—	2	1	2	7	8	1	24	30	31	11	4	121
K12/48/6 : 59	—	1	—	—	1	1	—	6	1	3	22	17	19	—	1	72
K14/48/8 : 18	1	1	1	—	—	—	—	3	7	1	18	23	20	11	1	87
1102 : 9	—	—	—	2	—	—	6	8	3	27	57	42	11	2	—	158
Total:	1	2	1	2	3	2	8	24	19	32	121	112	81	24	6	438

The percentage of univalents is here 32 (1386 out of 4380). Of these about 60 per cent were oriented on the plate. In early anaphase I from 0—8 accessories lagged between the poles with a mean value of 3,4. In late anaphase I—telophase I, from 0—5 were still seen in division. In late telophase I, however, accessories outside the nuclei were only seldom seen. At metaphase II non-oriented accessory chromosomes occur in different numbers in different plants, in one ranging from 0—5 with a mean value of 0,89, in another from 0—6 with a mean value of 2,00. In early anaphase II 2—3—4 accessory chromosomes are lagging between the poles, but most of them are included in the nuclei, and at late anaphase II—telophase II only 25—30 per cent of the cells show lagging accessory chromosomes. The number of tetrads with observable micronuclei was surprisingly low, being about 10 per cent. None of the four plants with ten accessories showed the earlier mentioned difficulties of separation of univalents at anaphase I.

Such anaphase I separation difficulties of univalents were found in

TABLE 9. *Late anaphase I—telophase I in a plant with 2n=14+11 st. acc.*

Number of cells with respective number of dividing univalent acc. chr. between the poles											Cells with bridges probably leading to restit. nuclei	Restit. nuclei	Number of cells
0	1	2	3	4	5	6	7	8	9	10			
185	36	12	7	5	5	2	3	8	—	1	6	18	288

a plant with $2n=14+11$ acc. Here the dividing univalents sometimes form so many bridges that a separation of the two daughter nuclei is not possible and a restitution nucleus is formed (Figs. 30—31). The result of an analysis of 288 late anaphase-telophase nuclei illustrates this point (Table 9).

The restitution nuclei, which have the unreduced number of chromosomes, enter a second metaphase and anaphase and give rise to dyads. The frequency of dyads calculated from the data above should be less than 10 per cent, but scored at the tetrad stage the dyads amount to 17 per cent, and the percentage of diploid pollen thus about 9.

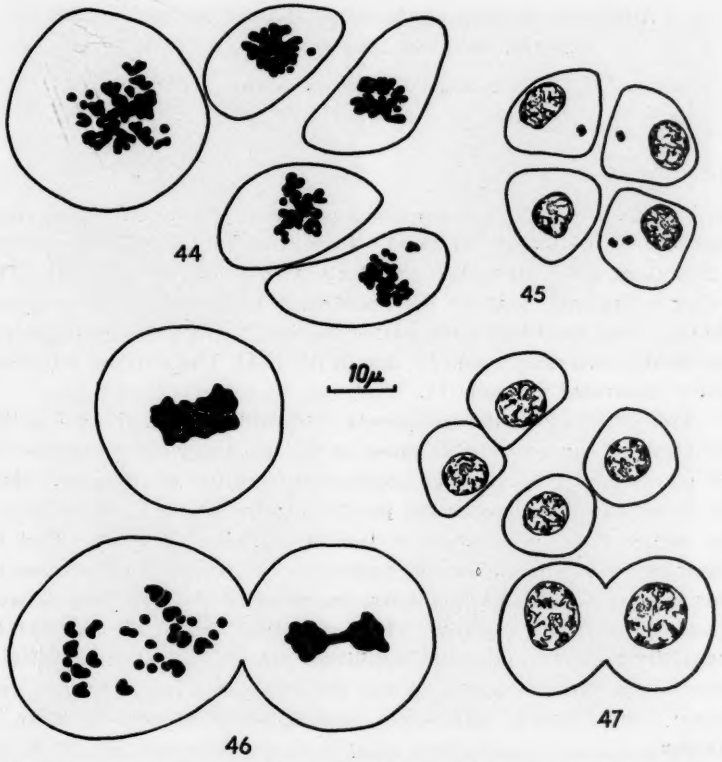
In a plant with fourteen accessory chromosomes the number of dividing univalents at anaphase I in 54 cells varied between 1—9 with a mean of 5,6. This led to a high number of restitution nuclei, not only in anaphase I but sometimes also at anaphase II, resulting in monads and triads (Table 10). Some tetraploid pollen mother cells indicating pre-meiotic restitution nuclei were also seen.

In this plant some of the normal bivalents had difficulties in separating at first anaphase; 12 out of 54 cells showed delayed separation of one or two bivalents. Whether this has anything to do with the presence of a high number of accessory chromosomes is not known.

Finally, one plant with fifteen and one with sixteen acessory chromosomes have been analysed. The plant with fifteen accessories had a high frequency of apparent multivalents at metaphase I, indicating sticky accessories, and at anaphase I bridges were often formed between dividing univalents with restitution nuclei as a result. The number of dividing accessory chromosomes in early anaphase I varied between 2—9 (mean=4,6), and at late anaphase I—telophase I 89 out of 252 cells

TABLE 10. *Analysis of the tetrad stage in plant 1014: 37 with 2n=14+14 st. acc.*

Tetrads	Triads	Dyads	Monads	Total number
180	5	84	2	271



Figs. 44—47. Later stages in meiosis in the plants with fifteen and sixteen acc. chr. — Fig. 44, Metaphase II in cells with reduced and unreduced number of chromosomes. The plant with $2n=14+15$ st. acc. — Fig. 45, Tetrads with micronuclei in the plant with $2n=14+16$ st. acc. — Figs. 46—47, Different stages in the formation of dyads and diploid pollen grains. The plant with $2n=14+15$ st. acc.

showed restitution nuclei. The frequency of dyads and triads is seen in Table 11. In this plant a remarkably low number of the tetrads and dyads had micronuclei.

In the plant with sixteen accessory chromosomes one never saw any bridges at late anaphase I or telophase I although it had a high number of dividing univalents at anaphase (5,6 in 105 cells with a variation from 1—9). The accessory chromosomes were generally all included in the nuclei at the latter stage. At late anaphase II, however, accessory chromosomes outside the nuclei were quite often seen, and

TABLE 11. *Analysis of the tetrad stage in the plants with 2n=14+15 st. acc. and 2n=14+16 st. acc.*

Number of acc. chr.	Tetrads	Triads	Dyads	Total number
15	129	3	89	221
16	250	—	—	250

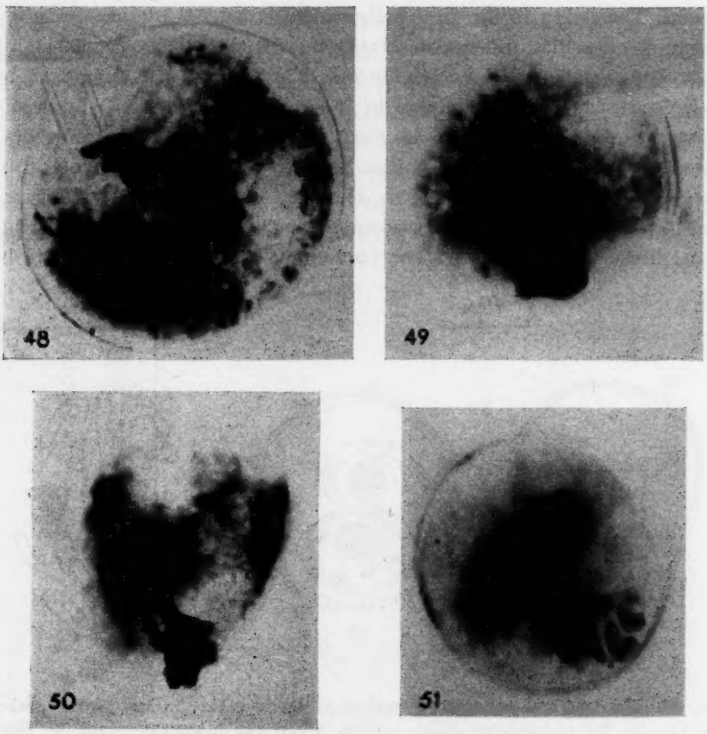
the plant had a fairly high frequency of micronuclei at the tetrad stage. Thus, out of 107 tetrads 46 had 1—4 micronuclei. Dyads were not seen in this plant although looked for in a very great number of cells. This is very interesting, because the plant with 15 accessory chromosomes and this plant were half sister plants, having the same maternal parent, and were grown nearly side by side in the field. The striking difference is best illustrated in Table 11.

The difficulty of the univalents in dividing at anaphase I is thus probably not caused by differences in the accessory chromosomes; the two plants having to a degree accessories from the same mother plant, nor in different environmental conditions; the plants growing only a few meters from each other in the field. It is more likely that the physiology of the host plant determines the behaviour of the accessories. This may serve as an explanation for most of the variation between plants in different respects. This, of course, does not exclude the possibility that environmental conditions can also affect the cytological behaviour of the accessories, or that the accessories can at least to some degree have inherent differences leading to differences in their behaviour.

V. POLLEN MITOSIS.

A similar type of directed non-disjunction of the accessory chromosomes in the pollen grain previously described in *Sorghum* (DARLINGTON and THOMAS, 1941), rye (MÜNTZING, 1946 a), *Anthoxanthum* (ÖSTERGREN, 1947), and maize (ROMAN, 1947, 1948, 1950) has been found to be at work in *Festuca* as well (BOSEMARK, 1950). In *Sorghum* the non-disjunction takes place in supernumerary divisions of the vegetative nucleus caused by the accessory chromosomes. In rye, *Anthoxanthum* and *Festuca* the accessories undergo non-disjunction at the first pollen mitosis, whereas in maize the non-disjunction takes place in the second division and is combined with directed fertilization.

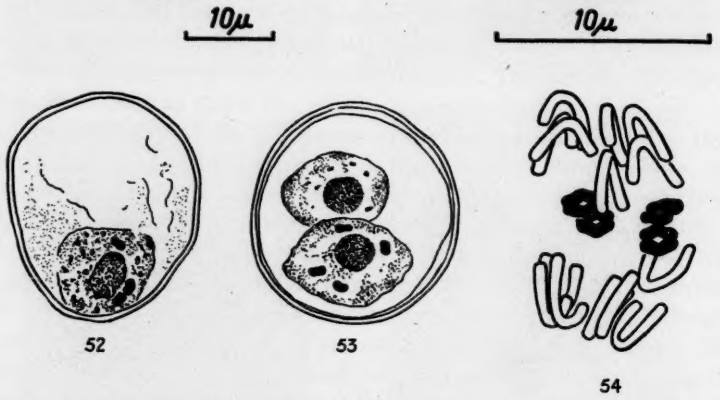
The mechanism in *Festuca pratensis* is easily studied cytologically in plants with a low number of accessory chromosomes. At the first



Figs. 48—51. The first pollen mitosis in plants with accessory chromosomes of the standard type. — Figs. 48—49, First anaphase in a plant with seven acc. chr. showing non-disjunction of the accessories. — Figs. 50—51, Disturbances caused by a high number of acc. chr. — Fig. 50, Anaphase, the chromosomes stick together and form bridges. — Fig. 51, Interphase with bridge formation (1500×).

pollen mitosis in these plants the accessories lag between the separating anaphase groups. In side view they have a characteristic appearance, similar to small bivalents (Figs. 48—49, 54). The centromere divides quite normally and pulls the chromatids apart, but these generally do not separate as they are held together near the ends. Sometimes it appears as though the attachment points could not resist the pull from the centromere and the chromatids fall apart. In most cases, however, both of the chromatids are included in the nucleus situated close to the pollen grain wall, the generative nucleus. In plants with up to four accessory chromosomes the first pollen mitosis in other respects seems quite

normal. The delay in the first pollen mitosis caused by accessory chromosomes described in maize (DARLINGTON and UPCOTT, 1941), rye (MÜNTZING, 1946 a) and *Anthoxanthum* (ÖSTERGREN, 1947) has not been subjected to a careful analysis in this material, but my observations indicate a similar situation in *Festuca*. With increasing numbers of accessories the number of pollen mitoses with sticky chromosomes, accessory as well as normal ones, increases correspondingly. This leads to a frequent clumping of the chromosomes at metaphase and to bridges at anaphase (Figs. 50—51). The frequency of bridges at late anaphase



Figs. 52—53. The heterochromatic nature of the acc. chr. in the pollen grain. — Fig. 52, At the uninucleate stage. — Fig. 53, At the binucleate stage. — Fig. 54, Drawing of the first anaphase in the pollen grain in a plant with seven acc. chr. Here it can be seen that the daughter chromosomes are held together close to the ends of the arms.

in 36 plants with varying numbers of accessories has been scored in 1951 and 1953 (Fig. 55).

From the diagram it can be seen that in plants with more than eight accessory chromosomes generally 50 per cent and more of all anaphases are disturbed. It is highly probable that the described phenomenon, together with formation of diploid and abnormal pollen grains, accounts for at least a part of the reduced pollen fertility found in plants with high numbers of accessory chromosomes (BOSEMARK, unpublished) and thus helps to limit the numerical increase of acccessory chromosomes in a population. In rye, where non-disjunction of accessory chromosomes takes place on both the male and female side, HÅKANSSON (1948) found that in plants with higher numbers of accessory chromo-

somes the first mitosis in pollen grains as well as embryo-sacs were frequently abnormal. Here the primary cause of the disturbances observed seems to be an inactivation of the spindle mechanism, whereas in *Festuca* it looks more like general stickiness. Owing to the relationship between heterochromatin and sticky properties the abnormalities observed in *Festuca* may be caused simply by the excess of heterochromatin, especially as the heterochromatic nature of the accessories is clearly manifested also in the pollen grains. Fig. 52 thus shows a uninuclear

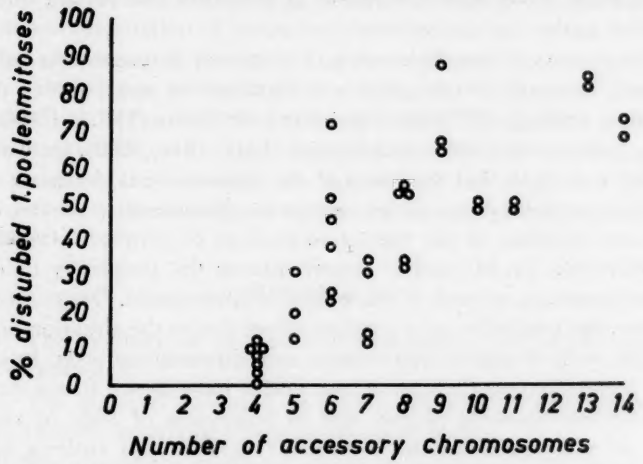


Fig. 55. The relation between number of accessory chromosomes present in a plant and number of disturbed 1. pollen mitoses.

pollen grain from a plant with seven accessories, Fig. 53 the binucleate stage in the same plant. In both cases the accessory chromosomes are easily distinguishable; in the binucleate pollen grain only in the generative nucleus. Only seldom do the accessory chromosomes form real micronuclei after the first pollen mitosis. The proportion of non-disjunctions can be calculated from the results of crosses where a mother plant lacking accessory chromosomes has been crossed with a plant having one or two of these chromosomes. In such crosses it has been shown that the frequency of non-disjunction varies highly between different plants. A full description of this will be given in a paper on the inheritance of accessory chromosomes in *Festuca*.

With respect to the small accessory chromosomes present in some plants, some undergo non-disjunction, others obviously have lost this

ability. It has not yet been possible to study these types at pachytene and to determine what part of the standard type they lack, and in this way get information on whether a special segment is responsible for the non-disjunction or not. Two of the large accessory chromosomes, which probably have a common origin, show a behaviour identical to the standard type at anaphase. In both of the cases standard accessory chromosomes were present together with the large ones, and a strict comparison could therefore be made. Also the large accessory chromosome appears to be heterochromatic at prophase and resting nuclei in the pollen grain.

The process of non-disjunction of accessory chromosomes has been discussed thoroughly in the papers of DARLINGTON and THOMAS (1941), MÜNTZING (1946 a), MÜNTZING and LIMA-DE-FARIA (1952), ÖSTERGREN (1947), HÅKANSSON (1948), and ROMAN (1947, 1948, 1950). DARLINGTON and THOMAS think that weakness of the centromere is the cause of the directed non-disjunction of the accessory chromosomes at the supernumerary divisions of the vegetative nucleus in *Sorghum*. ÖSTERGREN and MÜNTZING (in his earlier paper) discuss the possibility of differentiated segments located at the points of attachment. ÖSTERGREN also mentions the possibility of a position effect due to the situation of these segments with respect to centromere and chromosome ends. In a later paper MÜNTZING and LIMA-DE-FARIA (1952) have shown that a deficient accessory chromosome in rye, still in possession of both of the segments of attachment in the standard fragment, has entirely lost its capacity for non-disjunction. The lost part has a characteristic heterochromatic knob formation, and the authors suggest that this may have something to do with the non-disjunction mechanism. ROMAN (1947), studying translocations between B-chromosomes and normal ones in maize, demonstrated the existence of non-disjunction at the second division in the pollen grain. From different translocations involving smaller and larger parts of the B-chromosome he then arrived at the conclusion that the centromere was responsible for the non-disjunction. In a later report (ROMAN, 1950), however, he presents some evidence that the heterochromatic segment of the B-chromosome is also of importance for this process. Although the accessory chromosomes in the materials now presented are all heterochromatic (less markedly so in rye), it is not necessarily their heterochromatic nature alone which is the cause of non-disjunction. Thus, FERNANDES (1948) found that a heterochromatic supernumerary chromosome in *Narcissus Bulbocodium* divided quite normally at the first pollen mitosis. It therefore seems as

if the most probable explanation is that a specialized segment, heterochromatic or not, together with a modified centromere region under the special environmental conditions existing in the pollen grain at the time of the first division, is responsible for non-disjunction. Recently, LIMA-DE-FARIA (1953) studying the regions of special division cycle of *Agapanthus* chromosomes, has found that the region of the chromosome that holds together the two sister chromatids from early pro-metaphase II until anaphase II is not the kinetochore but the proximal regions of the arms. This makes it possible to assume this phenomenon and the non-disjunction of accessory chromosomes as related.

The asymmetry of the spindle at the first pollen mitosis is a well-known phenomenon in many genera, including the grasses (GEITLER, 1935; SAX and HUSTED, 1936; ÖSTERGREN, 1947). ÖSTERGREN points out that this must have a strong bearing on the directed non-disjunction where accessory chromosomes are included in the generative nucleus. The observations in the present material are entirely in agreement with this theory.

SUMMARY.

(1) The cytology of accessory chromosomes in *Festuca pratensis* HUDS. was studied.

(2) The material consisted of plants collected in natural populations in Sweden or obtained by crossing such plants.

(3) The size of the standard accessory chromosome is about $^1/_4$ of the mean for the normal chromosomes.

(4) Derivatives of this standard type were found in low frequencies, especially in crosses, and were designated small and very small accessory chromosomes. Still another type, about double the size of the standard accessory chromosome, the origin of which is not yet understood, was called large accessory chromosome.

(5) The accessory chromosomes in *Festuca pratensis* are highly heterochromatic in resting nuclei and prophase in mitosis and at prophase in meiosis.

(6) The position of the standard accessory chromosome in somatic metaphase plates is more central than that of the normal chromosomes.

(7) The number of at least the standard acccessory chromosome is highly constant in different parts of the plant.

(8) No pairing between accessory chromosomes and normal ones occurs at meiosis.

(9) At pachytene the accessory chromosomes form bivalents of a characteristic shape.

(10) The pairing of the accessories at later stages depends on the number present, but varies also between plants with the same number of accessory chromosomes.

(11) Univalent accessory chromosomes divide at anaphase I; in some plants with high numbers of accessories, difficulties in the separation of dividing univalents at this stage lead to the formation of restitution nuclei.

(12) The meiotic elimination of accessory chromosomes is surprisingly low even if a high number of univalents dividing at anaphase I are present.

(13) The accessories have the ability to undergo directed non-disjunction at the first pollen mitosis.

(14) With increasing numbers of accessory chromosomes an increasing number of the first divisions in the pollen grains are disturbed.

(15) There are indications that differences in the internal environment provided for the accessory chromosomes by their host plants are to a high degree responsible for the fairly big variation in behaviour of the accessories at meiosis and pollen mitosis.

LITERATURE CITED.

1. BOSEMARK, N. O. 1950. Accessory chromosomes in *Festuca pratensis* HUDS. — Hereditas XXXVI: 366—368.
2. BÖCHER, T. W. 1950. Chromosome behaviour and syncyte formation in *Phleum phleoides* L. KARST. — Botaniska Notiser 1950: 353—368.
3. DARLINGTON, C. D. and THOMAS, P. T. 1941. Morbid mitosis and the activity of inert chromosomes in *Sorghum*. — Proc. Roy. Soc. London, B 130: 127—150.
4. DARLINGTON, C. D. and UPCOTT, M. B. 1941. The activity of inert chromosomes in *Zea mays*. — Journ. of Gen. 41: 275—296.
5. FERNANDES, A. 1948. Sur la repartition d'un hétérochromatinosome surnuméraire dans le pollen. — Bol. Soc. Broteriana XXII, 2 série: 119—137.
6. GEITLER, L. 1935. Beobachtungen über die erste Teilung im Pollenkorn der Angiospermen. — Planta 24: 361—386.
7. HEITZ, E. 1932. Die Herkunft der Chromozentren. — Planta 18: 571—635.
8. HÅKANSSON, A. 1945. Überzählige Chromosomen in einer Rasse von *Godetia nutans* HIORTH. — Botaniska Notiser 1945: 1—19.
9. — 1948. Behaviour of accessory rye chromosomes in the embryo-sac. — Hereditas XXXIV: 35—59.
10. — 1949. Supernumerary chromosomes in *Godetia viminea*. — Hereditas XXXV: 375—389.

11. HÅKANSSON, A. 1950. Spontaneous chromosome variation in the roots of a species hybrid. — Hereditas XXXVI: 39—59.
12. LIMA-DE-FARIA, A. 1948. B chromosomes of rye at pachytene. — Portug. Acta Biol. (A) 2 (3): 167—173.
13. — 1952. Chromomere analysis of the chromosome complement of rye. — Chromosoma 5 (1): 1—68.
14. — 1953. The regions of special cycle of division of *Agapanthus* chromosomes. — Chromosoma 6: 33—44.
15. LONGLEY, A. E. 1938. Chromosomes of maize from North American Indians. — Jour. Agr. Research 56: 177—195.
16. MCCLINTOCK, B. 1933. The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. — Z. f. Zellforsch. 19: 191—237.
17. MORGAN, D. T., JR. 1943. The formation of chromocenters in interkinetic nuclei of maize by knobs and B chromosomes. — J. of Hered. 34 (7): 195—198.
18. MYERS, W. M. and HILL, H. D. 1947. Distribution and nature of polyploidy in *Festuca elatior* L. — Bull. Torrey Bot. Club 74 (2): 99—111.
19. MÜNTZING, A. 1933. Apomictic and sexual seed formation in *Poa*. — Hereditas XVII: 131—154.
20. — 1944. Cytological studies of extra fragment chromosomes in rye. I. Iso-fragments produced by misdivision. — Hereditas XXX: 231—248.
21. — 1945. Cytological studies of extra fragment chromosomes in rye. II. Transmission and multiplication of standard fragments and iso-fragments. — Hereditas XXXI: 457—477.
22. — 1946 a. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at the pollen mitosis. — Hereditas XXXII: 97—119.
23. — 1946 b. Different chromosome numbers in root tips and pollen mother cells in a sexual strain of *Poa alpina*. — Hereditas XXXII: 127—129.
24. — 1948 a. Cytological studies of extra fragment chromosomes in rye. IV. The position of various fragment types in somatic plates. — Hereditas XXXIV: 161—180.
25. — 1948 b. Cytological studies of extra fragment chromosomes in rye. V. A new fragment type arisen by deletion. — Hereditas XXXIV: 435—442.
26. — 1948 c. Accessory chromosomes in *Poa alpina*. — Heredity II (1): 49—61.
27. — 1949. Accessory chromosomes in *Secale* and *Poa*. — Proc. Eighth Int. Cong. of Gen. (Hereditas, Suppl. Vol.): 402—411.
28. — 1950. Accessory chromosomes in rye populations from Turkey and Afghanistan. — Hereditas XXXVI: 507—509.
29. — 1954. Cytogenetics of accessory chromosomes (B-chromosomes). — Proc. Ninth Int. Cong. Genetics. (In the press.)
30. MÜNTZING, A. and LIMA-DE-FARIA, A. 1949. Pachytene analysis of standard fragments and large iso-fragments in rye. — Hereditas XXXV: 253—268.
31. — and — 1952. Pachytene analysis of a deficient accessory chromosome in rye. — Hereditas XXXVIII: 1—10.
32. PETO, F. H. 1933. The cytology of certain intergeneric hybrids between *Festuca* and *Lolium*. — Journ. of Gen. 28: 113—156.

33. RANCKEN, G. 1934. Zytologische Untersuchungen an einigen wirtschaftlich wertvollen Wiesengräsern. — Acta Agralia Fenn. 29: 1—92.
34. RANDOLPH, L. F. 1928. Types of supernumerary chromosomes in maize. (Abstract). — Anat. Rec. 41: 102.
35. — 1941. Genetic characteristics of the B chromosomes in maize. — Genetics 26: 608—631.
36. ROMAN, H. 1947. Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. — Genetics 32: 391—409.
37. — 1948. Directed fertilization in maize. — Proc. Nat. Acad. Sci. 34: 36—42.
38. — 1950. Factors affecting mitotic nondisjunction in maize. Abstract 1949. Meetings of the Genetics Society of America. — Genetics 35: 132.
39. SAX, K. and HUSTED, L. 1936. Polarity and differentiation in microspore development. — Am. J. Bot. 23: 606—609.
40. SPARROW, A. H., POND, V. and SPARROW, R. C. 1952. Distribution and behaviour of supernumerary chromosomes during microsporogenesis in a population of *Trillium erectum* L. — The Am. Nat. LXXXVI (830): 277—292.
41. TISCHLER, G. 1953. Allgemeine Pflanzenkaryologie, Band. II, Handbuch der Pflanzenanatomie, Bd. II. Erg.-Bd. Lf. 1.: 83—91. — Berlin-Nikolassee.
42. WHITE, M. J. D. 1935. The effects of X-rays on mitosis in the spermatogonial divisions of *Locusta migratoria* L. — Proc. Roy. Soc. London, B 119: 61—84.
43. — 1945. Animal cytology and evolution. — Cambridge.
44. ÖSTERGREN, G. 1947. Heterochromatic B-chromosomes in *Anthoxanthum*. — Hereditas XXXIII: 261—296.
45. ÖSTERGREN, G. and VIGFUSSON, E. 1953. On position correlations of univalents and quasi-bivalents formed by sticky univalents. — Hereditas XXXIX (Festskrift för ARNE MÜNTZING 2.3.1953): 33—50.

CONTENTS.

I. Introduction 346
II. Materials and methods 346
III. Mitosis 347
1. Morphology of the normal chromosome complement 347
2. Morphology and frequency of different accessory chromosomes 347
3. Heterochromatic nature of the accessory chromosomes 350
4. The position of the accessory chromosomes in somatic metaphase plates 351
5. Constancy of the accessory chromosomes within plants 353
IV. Meiosis 354
1. Plants without accessory chromosomes 354
2. Plants with accessory chromosomes 355
A. General behaviour of accessory chromosomes in meiosis 355
B. Meiosis in plants with one standard accessory chromosome 358
C. Plants with two standard accessory chromosomes 360
D. Meiosis in plants with higher numbers of standard accessory chromosomes 361
V. Pollen mitosis 368
Summary 373
Literature cited 374

INVESTIGATIONS ON NORTH AMERICAN CALAMAGROSTIS. I

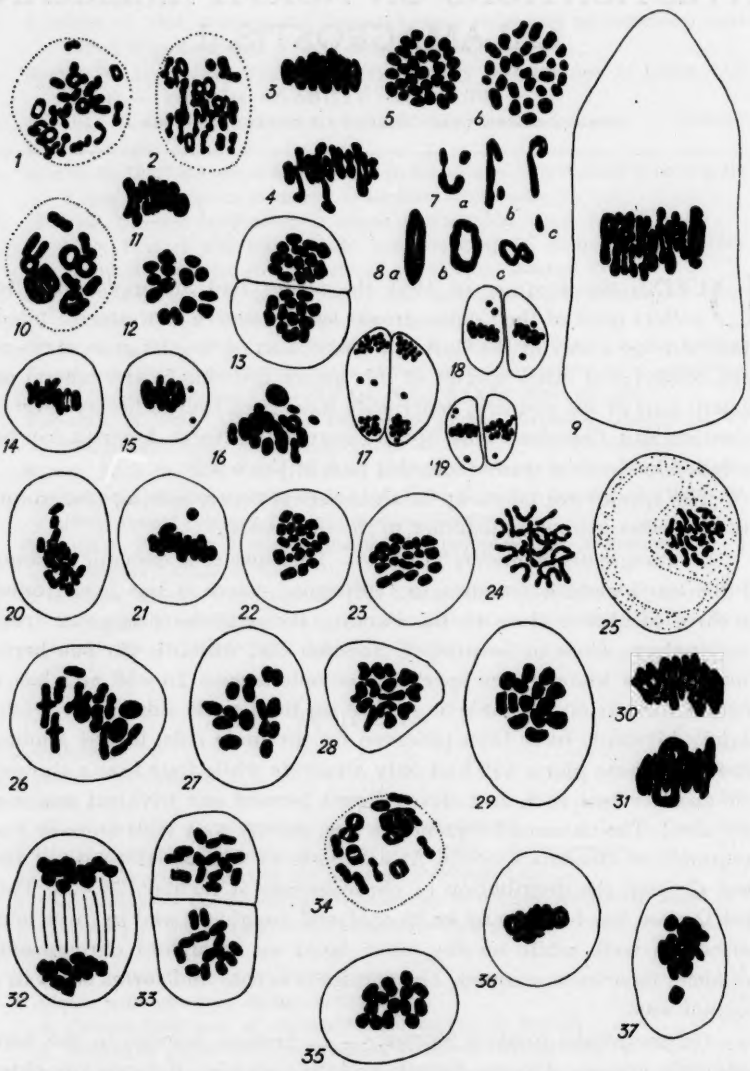
BY *AXEL NYGREN*
ROYAL AGRICULTURAL COLLEGE OF SWEDEN, UPPSALA

DURING the summer of 1951 the writer had an opportunity to collect most of the *Calamagrostis* species native to western United States during a stay at the Carnegie Institution of Washington at Stanford. Material of other species of this genus, growing in the central or eastern part of the country, generously have been sent to me by several scientists and, therefore, most species growing in North America can be included in the first study from this part of the world.

The species are taken up in alphabetical order, leaving the grouping of species belonging together to the discussion.

Calamagrostis bolanderi THURB. — This species is endemic to some of the northwestern counties in California, where it has been found in three localities, close to the Pacific. My material originates from Cunningham, close to Sevastopol, Sonoma Co., which is the southernmost locality known. The species is octoploid with $2n=56$ and has a regular meiosis on the male as well as on the female side (Figs. 1—9). Mainly bivalents have been observed on the male side, in 564 studied first metaphase plates 549 had only bivalents while four Pmc's showed one quadrivalent each and eleven Pmc's formed one trivalent and one univalent. The chiasma frequency is low, mostly rods with more or less terminalized chiasmata occur. At anaphase as well as telophase of the first division the distribution of chromosomes is regular, 28—28. This distribution has been found in 29 analysed anaphases and in three telophases as well, while on the other hand no aneuploid chromosome numbers have been counted. The species is sexual and forms seeds in a normal way.

Calamagrostis breweri THURB. — *C. breweri* belongs to the high mountain grasses of Sierra Nevada and the Cascades. It forms two chromosomal races with $2n=28$ and $2n=42$, respectively, which seem to be geographically separated, by a distribution limit going some miles south of Lake Tahoe. The tetraploid chromosome race has the southern and the hexaploid race the northern distribution area (*cf.* map, Fig. 94).



1—9, *C. bolanderi*. 1, diakinesis, 28 II; 2, diakinesis, III+26 II+I; 3—4, M I, side view; 5, M I, 28 II; 6, M I, 26 II+IV; 7, different types of III's; 8, different types of IV's; 9, the female side, M I with 28. II. — 10—19, *C. breweri*, tetraploid. 10, diakinesis, 14 II; 11, M I, side view, 14 II; 12, M I, polar view, 14 II; 13, M II, the distribution 14—14; 14—19, plant with accessory chromosomes. 14—15, M I; 16, pro-

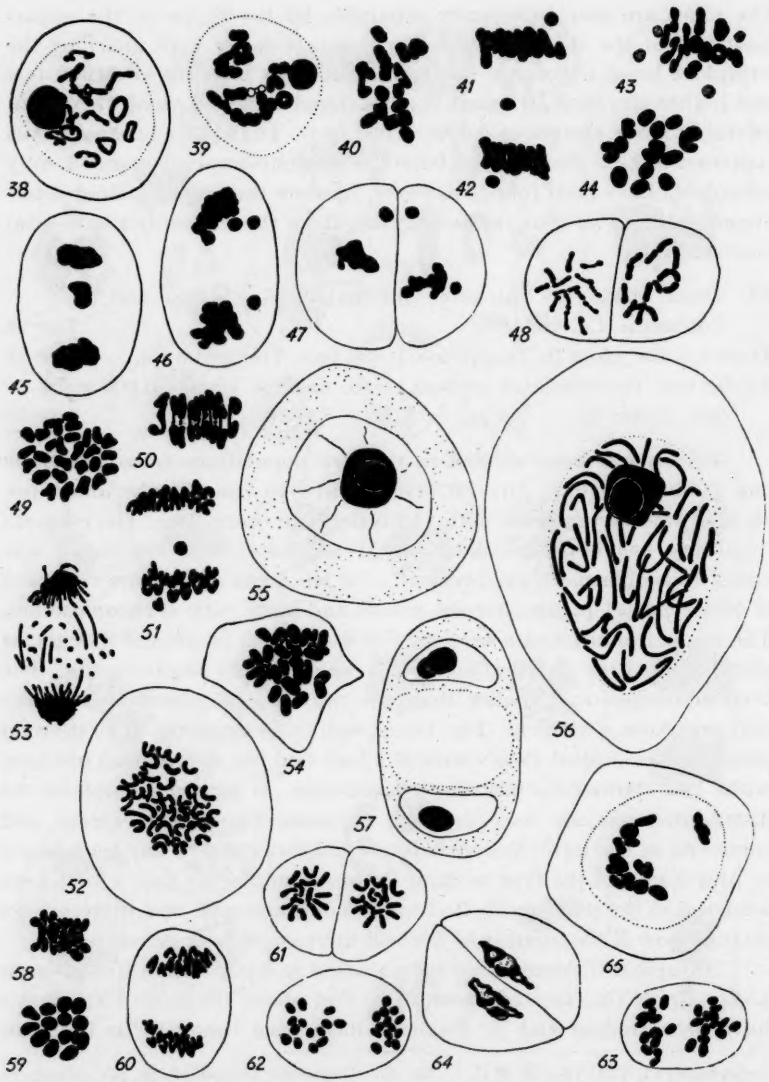
The races are morphologically separable by the shape of the uppermost leaf of the straw, ecologically they are easily kept apart by the tetraploid being a grass of the high mountains growing at 10.000 feet and higher elevation, whereas the hexaploid grows in woods at a considerably lower elevation (6.000—7.000 feet). The tender tetraploid was supposed to be a diploid type before it was chromosome counted, only tetraploids have been found, however, in more than one hundred determined plants. The tetraploids originate from three localities in central Sierra Nevada:

Mt. Dana, close to Yosemite National Park, 12.000 feet, Toulumne Co. ..... $2n=28$
Tenaya Lake, close to Tioga Pass, 9.000 feet, Toulumne Co. .. $2n=28$
Timberline experimental station, Hall natural area, 10.000 feet, Mono Co. ..... $2n=28$

Meiosis has been studied in the two populations from Mt. Dana and Timberline (Figs. 10—19). The population from Timberline (Figs. 10—13) was very regular with 14 bivalents in every Pmc. The chiasma frequency has been calculated in 54 analysed cells, the mean was $1{,}156 \pm 0{,}0133$ chiasmata per bivalent. The Mt. Dana population consisted of two types of plants, normal plants and such with B-chromosomes. The normal plants had a very regular meiosis, in 50 second metaphase plates studied the distribution 14—14 was the only one occurring. The B-chromosomes are smaller than the normal chromosomes and may pair and form a bivalent (Fig. 14) as well as be unpaired at first metaphase. In six studied Pmc's with M I four had the distribution one-one, while two showed the distribution two-zero. At second metaphase the distribution one-one was found in 17 cases, two-zero in eleven and three-one in one case. Meiosis with 14 bivalents at M I has been found in four Emc's of the type without B-chromosomes, no Emc's have been analysed in the plants with B-chromosomes, however, and therefore we do not know if the number of B's will increase in later generations.

Hexaploid *C. breweri* has been studied in a population from Woods Lake, Alpine Co., located about 7.000 feet above sea-level. Fifty Pmc's have been studied and 21 bivalents have been found to be the most

metaphase; 17, T II; 18—19, M II. — 20—25, *C. breweri*, hexaploid. 20, prometaphase, 21 II; 21, M I, III+19 II+I; 22—23, III+19 II+I; 24—25, pollen mitosis, 21 chromosomes. — 26—28, *C. densa*. 26, M I, 14 II; 27, prometaphase, 14 II; 28, M I, 14 II, polar view. — 29—33, *C. foliosa*. 29, M I, polar view, 14 II; 30, M I, 14 II; 31, IV+13 II; 32, A I; 33, M II. — 34—37, *C. howellii*. 34, diakinesis, 14 II; 35, M I, polar view, 14 II; 36, M I, side view, 14 II; 37, prometaphase, 14 II.



38—48, *C. koelerioides*. 38—39, diakinesis, 15 II (14 A+B); 40, prometaphase, 14 II+ +2 I; 41—42, M I, 14 II+2 I; 43, M I, 13 II+4 I; 44, prometaphase, 15 II; 45—46, A I, lagging chromosomes; 47, M II; 48, M II to the right and A II to the left. — 49—57, *C. canadensis*. 49, M I, polar view, 28 II; 50, just as 49, but side view; 51, A I; 52, mitotic division in a Pmc; 53, fragmented anaphase after mitotic division in a Pmc;

common configuration (Fig. 20). In three cases, one III, 19 II and one I have been observed (Figs. 21—23). A great number of pollen mitoses have been analysed, in all cases 21 chromosomes have been counted in the cells (Figs. 24—25). The Emc develops normally into a tetrad, the chalazal cell of which develops the ES. Thus, tetraploid as well as hexaploid *C. breweri* are sexual species with a way of reproduction which is similar to other sexuals of the genus.

Calamagrostis cainii HITCHC. — This species is endemic to Mount LeConte, Smoky Mnts., Tennessee; the author is indebted to Dr. AARON SHARP, Knoxville, for living material. *C. cainii* is a tetraploid with $2n=28$ which forms good pollen and develops an ES according to the normal scheme. Although no closer studies have been made as yet of meiosis it can be classified as a sexual species.

Calamagrostis californica KEARNEY. — This »species» which was collected for the first time by LEMMON in Sierra Nevada in 1875 and later on not has been found again with certainty was discovered by the present author close to Tioga Pass in Sierra Nevada at an elevation of 10.000 feet. It is nothing but a tall polyploid form of *C. inexpansa* ($2n=84$) with exceptionally big spikelets, and will therefore be taken up together with *inexpansa* later on in this paper.

Calamagrostis canadensis (MICHX.) BEAUV. — *C. canadensis* constitutes the western part of a vast form-circle formed by *C. purpurea* in Europe, *C. Langsdorfii* in northern Asia and *C. canadensis* in North America. Ecologically *canadensis* is very closely related to *C. purpurea*, morphologically they generally are easy to hold apart. The material studied by the author has mainly been collected by colleagues in the United States and in Canada. The chromosome numbers found are the following:

Maine, Mt. Washington, close to the base of the mountain (No. 24)	$2n=42$ (10 pl.)
Maine, Mt. Washington (No. 1609)	$2n=42$
New York, Ithaca, swamp S of the airport (No. 1605)	$2n=45$
New York, Ithaca, $^1/_2$ mile N of Butterwith Falls (No. 1604)	$2n=42, 56$
Maine, Mt. Washington, firs summit (No. 29)	$2n=48, 49, 51$
California, Timberline, Mono Co., 10.000 feet	$2n=56, 56, 56$

54, M I in the Emc; 55; diplosporous resting nucleus; 56, mitotic division in the Emc; 57, a dyad formed after mitotic division. — 58—65, *C. inexpansa*, $2n=28$. 58—59, M I, 14 II; 60—63, A I, normal; 64, formation of restitution nuclei during T II; 65, diakinesis in the Emc.

Canada, Montreal Bot. Garden (No. 41) ..... $2n=56$ (15 pl.)
Minnesota, N shore of Lake Superior (No. 5) ..... $2n=62$ (10 pl.)
Maine, Mt. Washington, close to the base of the mountain (No. 25) ..... $2n=65, 66$ (8 pl.)
C. canadensis var. *robusta*
Canada, Ontario, Thunder Bay (No. 19) ..... $2n=42$ (20 pl.)

The variation in chromosome number of *C. canadensis* is not so great as in *C. purpurea* ($2n=56$—91) but on the other hand a lower number of populations from nature have been studied in *canadensis* than in *purpurea* (over 200).

Cytological studies have been made of meiosis on the male as well as on the female side of the material from Timberline, Montreal and Minnesota (Figs. 49—57). The strain from Montreal has a regular meiosis in the Pmc's as well as in the Emc's (Figs. 49—51, 54); in the anthers only bivalents are formed (80 Pmc's tested). The Emc goes through a regular meiosis (Fig. 54) and forms a tetrad, the chalazal cell of which develops the ES. The type from Minnesota is almost as regular as the Montreal *canadensis*, but now and then shows univalents in the male meiosis. Thus, ten out of 110 tested Pmc's held one univalent per cell. All these ten Pmc's also had one trivalent. The formation of polyvalents is low, except the trivalents mentioned only one single quadrivalent has been observed in the Pmc's studied. The first anaphase is just as regular as M I, only two Pmc's out of 25 held lagging chromosomes and in 70 first telophase cells studied not a single univalent has been found. The Minnesota type is diplosporous, although it is able to form meiosis on the female side. The Timberline type is of particular interest because of the fact that it has only mitotic division on the male as well as on the female side. In the loculi the Pmc's rest for a long time before they begin to divide, when the division starts the chromosomes are unpaired, long and slender and undoubtedly are of mitotic type (Figs. 52—53). In this respect the Timberline *canadensis* behaves just as a particular type of *C. purpurea* earlier described (NYGREN, 1946, pp. 159—161). In the *purpurea* case transitional forms between chromosomes of a more contracted, meiotic-like appearance and such of a slender mitotic shape occurred. In *canadensis*, on the contrary, only true mitotic chromosomes have been found. In the *purpurea* strain the anthers of the first panicles developed on the plant had a semiheterotypic division followed by pollen formation, whereas panicles later developed only produced mitotical divisions in the Pmc's

followed by the formation of plasmodia in the loculi. In the *canadensis* strain no pollen formation at all occurs, all Pmc's degenerate after the formation of plasmodia of different sizes. In some cells the mitotic chromosomes are very slender in the metaphase stage and in the following anaphase many chromosomes are fragmented. These anaphase cells have an appearance similar to such cells which have been exposed to radiation (Fig. 53). On the female side diplospory with mitotic division occurs, *i. e.* the same type which has been stated for European *Calamagrostis* species by the present author (NYGREN, 1946). Thus, the Emc has a long resting stage (Fig. 55), which is followed by a very rapid mitotic division (Fig. 56). The later development of the Emc follows the *Antennaria* scheme, and thus no fertilization of neither egg cell nor central nucleus is needed for the formation of seeds. It is clear from the data above that *C. canadensis* is a semiapomictic species which behaves in the same way as *C. purpurea* as to the formation of new forms.

Calamagrostis crassiglumis THURB. — This species, which obviously is related to *C. inexpansa*, occurs along the west coast of North America from Vancouver Island to central California. The author has collected his material close to the Radio station at Point Reyes, Marin Co., Cal., in a swamp about 300 m. from the Pacific. The species has the highest chromosome number known in *Calamagrostis* up to now: $2n=140$. The formation of pollen and seeds has not been studied as yet because of the difficulties to obtain flowering material.

Calamagrostis densa VASEY. — *C. densa* is endemic to southernmost California, but might also occur in Mexico. The species grows on dry hills in S. Diego Co., at an elevation of about 5.000 feet. Because of the restriction according to the danger of fire in this area in the summer of 1951, the author was obliged to try to find the species in a new locality to be able to obtain living material. All five localities earlier known were namely located in areas not allowed to enter. The new locality is situated along Highway 80, close to Descano, at an elevation of about 5.000 feet. The species is tetraploid with $2n=28$ and forms a regular meiosis with 14 bivalents on the male as well as on the female side (Figs. 26—28). The chiasma frequency has been determined in 51 cells, the mean average has been calculated to be $1{,}191 \pm 0{,}028$ chiasmata per bivalent.

Calamagrostis fernaldii LOUIS-MARIE. — This species is endemic to Boarstone Mnts., Piscataquis Co., Maine; the author has, however, had no opportunity to visit this remote locality, but Professor F. H.

STEINMETZ and his colleague Dr. OGDEN kindly have handed material over to me, and I am very indebted to these gentlemen for sending me this material. The species is a dodecaploid with $2n=84$, which shows many similarities to certain *inexpansa* biotypes. Meiosis has not been studied as yet, but the species forms good pollen. Tetrads have been observed as a result of divisions of the Emc, and thus the species very probably is a sexual species.

Calamagrostis foliosa KEARNEY. — *C. foliosa* is endemic to a small part of the coast of Humboldt and Mendocino Counties in northern California, where it grows on steep rocks exposed to the Pacific. My material is collected one mile north of Westport, along Highway 1, Mendocino Co. The species is tetraploid with $2n=28$ and has a regular meiosis on the male as well as on the female side (Figs. 29—33). The chiasma frequency has been calculated in 62 Pmc's, the average number of chiasmata per bivalent is $1{,}233 \pm 0{,}012$. One occasional quadrivalent has been found (Fig. 31). The Emc goes through a regular meiosis and forms a tetrad, the chalazal cell of which gives rise to the ES. The species no doubt is sexual.

Calamagrostis howellii VASEY. — *C. howellii* is endemic to the Columbia river area E of Porland, where the species grows on both sides of the river. My material originates from Multnomah Falls and Latourell Falls in northern Oregon. The species is tetraploid with $2n=28$ and has regular meiosis on the male as well as on the female side (Figs. 34—37). The chiasma frequency has been calculated in 44 Pmc's, the average per bivalent is $1{,}233 \pm 0{,}012$. All diakinesis and first metaphase plates studied showed 14 bivalents, no quadrivalents have been observed. On the female side meiosis followed by formation of tetrads has been found. The species has sexual seed production.

Calamagrostis inexpansa A. GRAY. — *C. inexpansa* is a polyploid species with several chromosome races. Some populations have been named separately, thus *C. californica* is an *inexpansa* with big spikelets and very broad-ridged leaves. The species is closely related to *C. neglecta*, which always is tetraploid, however. The chromosome numbers found in the *inexpansa* populations studied are the following:

California, Tioga Pass, Mono Co., 10.000 feet elevation ..... $2n=28$
California, Tioga Pass, Mono Co., 10.000 feet elevation, *C. californica* ..... $2n=84$
Minnesota, Ottertail Co. ..... $2n=56, 58, 58$

California, Alkaline bog, Timberline, Mono Co., 10.000 feet elevation ..... $2n=84$

Colorado, Shore of Muskee Lake, Boulder Co., 9.000 feet elevation ..... $2n=85, 88, 89, 92, 98, 103, 105$

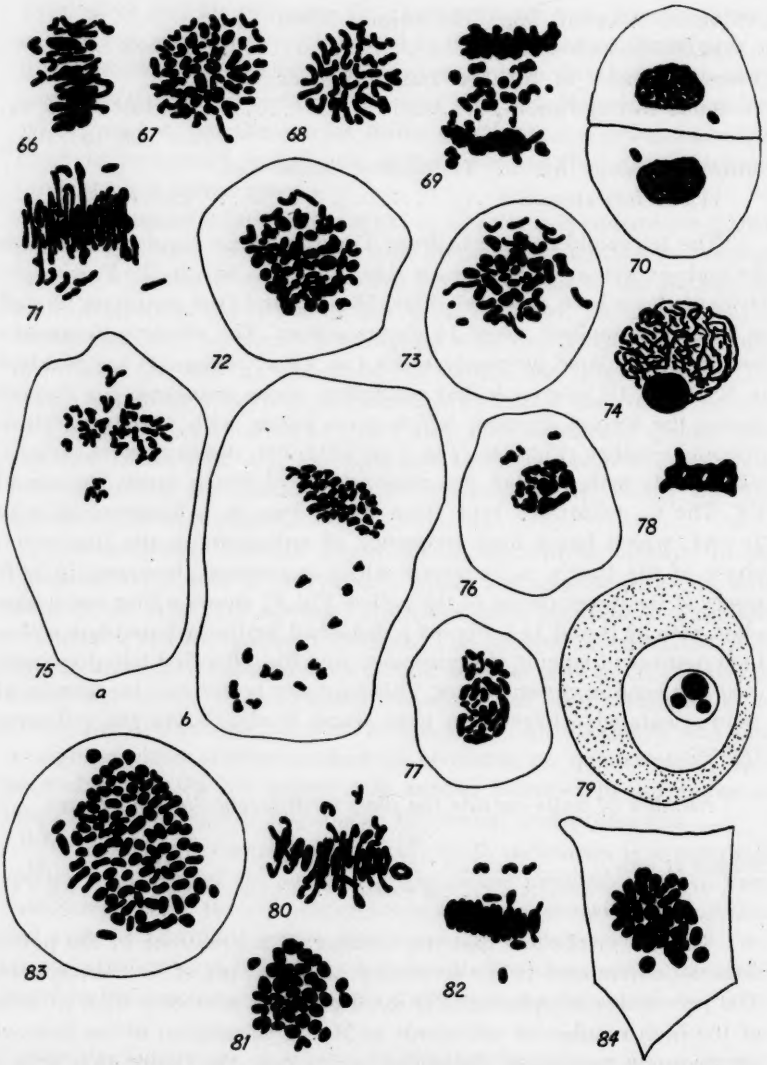
California, Slope SE of Timberline, Mono Co., 11.000 feet elevation ..... $2n=102$

The tetraploid *inexpansa* from Tioga Pass has regular meiosis on the male as well as on the female side (Figs. 58—65). In the Pmc's only bivalents have been observed (Figs. 58—59) and first anaphase as well as second metaphase show 14 chromosomes. The chiasma frequency has been calculated averagely to be $1{,}234 \pm 0{,}011$ chiasmata per bivalent in 50 Pmc's. Of interest is that restitution nuclei sometimes are formed during the second division, which gives pollen with unreduced chromosome number (Fig. 64). The Emc (Fig. 65) shows normal meiosis, which ends with a tetrad, the chalazal cell of which forms the sexual ES. The *C. californica* type from Tioga Pass is a dodecaploid with $2n=84$, which has a high frequency of univalents in the first metaphase of the Pmc's, a frequency which is reduced, however, in later stages of the development of the pollen. Fig. 67 shows a first metaphase plate with 35 II and 14 I, Fig. 69 a disturbed first anaphase plate with a high number of lagging chromosomes, and Fig. 70 a first telophase with only two lagging chromosomes. This tendency to decrease the number of »non-orientated» elements in later stages is clear from the following values:

Number of units outside the plate in different dividing stages.

	M I	A I	T I	T II
Number of cells	80	52	116	61
» » units	$7{,}01 \pm 0{,}244$	$4{,}71 \pm 0{,}48$	$0{,}80 \pm 0{,}09$	$0{,}30 \pm 0{,}09$

Thus, it is obvious that the number of micronuclei in the pollen does not correspond to the frequency of univalents at first metaphase. The percentage morphologically good pollen is also very high in spite of the high number of univalents at M I. A calculation of the average chromosome number of the pollen grains gave the figure $43{,}56$ with a variation in number of 16 cells between 38 and 49. On the female side only meiosis has been found. The first metaphase shows as high a univalent frequency as the Pmc's (Fig. 71). The *californica* strain of *inexpansa* obviously is sexual.



66—79, *C. inexpansa* including *C. californica*. 66, M I in the Alkaline bog type; 67, *C. californica*, 35 II+14 I; 68, reduced cell with 31 chromosomes only; 69, A I, disturbed; 70, T I with two laggards only; 71—79, the female side; 71, Emc in *C. californica*, M I; 72, the same in Alkaline bog type; 73, Emc in the Timberline type; 74, Alkaline bog, formation of a restitution nucleus at prophase; 75 *a* and *b*, Alkaline

The type from Alkaline bog also has the chromosome number $2n=84$. This strain shows the same tendency as *californica* in decreasing the number of univalents and their derivatives in later division stages. The number of univalents at first metaphase as an average of one hundred analysed Pmc's was $2{,}12 \pm 0{,}15$, while the corresponding figures for one hundred first telophase plates was $1{,}91 \pm 0{,}16$ and that for 35 second telophase plates $0{,}23 \pm 0{,}09$. Thus, the number of units is lower in the Alkaline bog type than in *californica* (Fig. 66). The female side of this strain is of great theoretical interest. In some nucelli the Emc has a normal meiosis, the first metaphase of which, however, may show a considerable number of univalents (Fig. 72). In other nucelli, however, the bivalents do not divide at first metaphase but form a restitution nucleus (Figs. 76—78), which later on will give rise to unreduced ES's. Sometimes the formation of the restitution nucleus begins already at pachytene (Fig. 74), whereas in other cases the chromosomes go through a first metaphase, but form a restitution nucleus during the anaphase stage in such a way that some of the chromosomes go to one of the poles, while all the remaining ones fuse and form the restitution nucleus (Fig. 75 *a* and *b*). The last case gives rise to a restitution nucleus with somewhat reduced chromosome number. The Alkaline bog type no doubt shows transitional stages between normal meiosis and diplospory.

The last type studied is the strain from the SE slope at Timberline with $2n=102$. Even here the number of univalents and their derivatives decrease in later division stages. The average univalent frequency of 101 analysed Pmc's is $3{,}43 \pm 0{,}33$ at first metaphase, while there are $2{,}11 \pm 0{,}19$ lagging chromosomes at first anaphase. In some Emc's a disturbed meiosis has been found, whereas other Emc's are diplosporous and develop according to the *Antennaria* scheme (Fig. 79). Therefore, the Timberline type of *inexpansa* is a semiapomictic strain.

Calamagrostis koelerioides VASEY. — This species, which occurs even outside California, is closely related to *C. densa* and grows, just like the latter, on dry hills, preferably on serpentine. The material available to the author originates from Pulga in Butte Co. in central California and was generously collected and handed over to me by Dr. THOMAS HOWELL of San Francisco. Two plants were collected, which

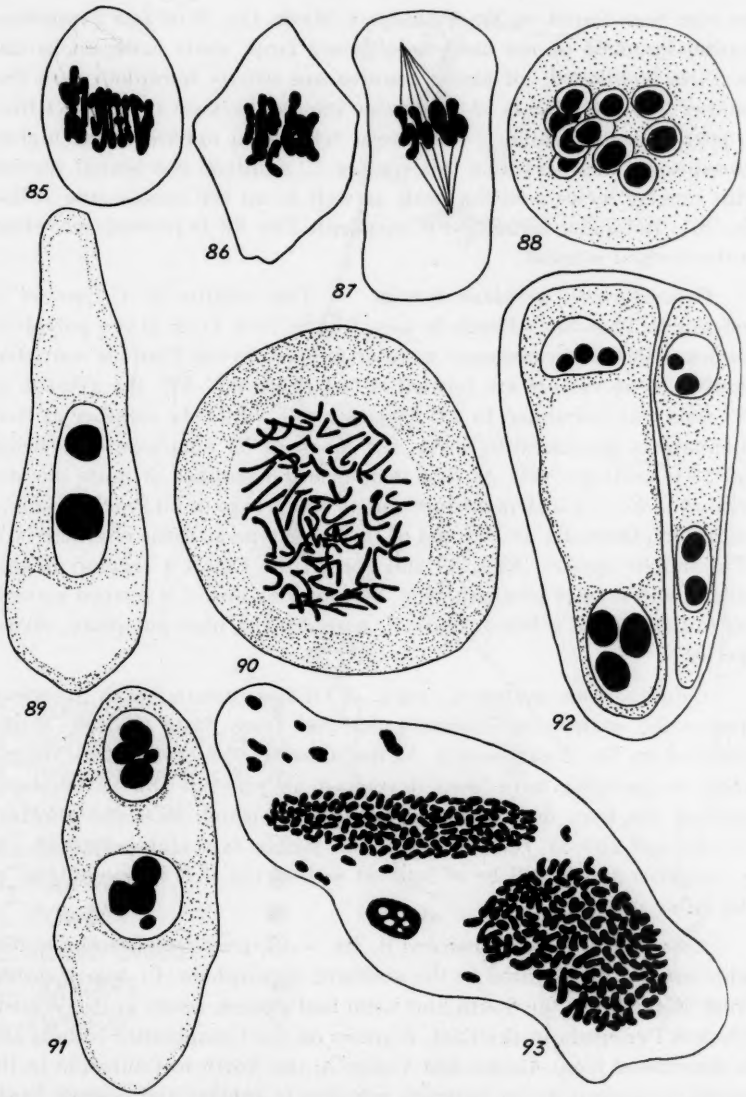
bog, formation of a restitution nucleus at A I, 14 chromosomes lagging; 76—78, different ways to form restitution nuclei in the Alkaline bog type; 79, resting stage ending with a mitotic division. — 80—84, *C. perplexa*. 80, M I, side view; 81, M I, polar view; 82, M I, side view; 83, plasmodium formed by several Pmcs; 84, Emc with regular pairing at M I.

have the chromosome numbers $2n=28$ and $2n=30$, respectively. Meiosis of the tetraploid is quite regular, while that of the 30 chromosomal form is disturbed (Figs. 38—48). The two supernumeraries sometimes form a bivalent (Figs. 38, 39, 44), but in most cases the two chromosomes are unpaired (Figs. 41—42), which causes lagging chromosomes at first anaphase as well as at telophase (Figs. 45—46). In 137 out of 442 M I cells studied the supernumeraries formed a bivalent (=31 %) whereas distance conjugation was observed in 22 cells (=4,98 %). In 58 out of 146 A I plates the supernumerary chromosomes were lagging (39,73 %). In 5,2 % of the Pmc's quadrivalents were observed. In some cases pollen is formed with the chromosome number $n=15$ and, therefore, the aneuploid number may stabilize in later generations (Fig. 48). Meiosis in the Emc is regular and the ES formation follows the normal scheme. *C. koelerioides* no doubt is a sexual species.

Calamagrostis montanensis SCRIBN. — Of this central North American species the author has obtained material partly from Boulder, Colorado, by Dr. WEBER, partly through exchange of seeds with the Botanical Garden at Ottawa, Canada. Two collections have been obtained from Ottawa, one originating from Cardstone, Alberta, the other one without information of the locality. The chromosome number of all plants raised from the seeds obtained was $2n=28$, and thus *C. montanensis* constitutes another tetraploid of the New World. The species is sexual and forms excellent pollen.

Calamagrostis nutkaensis (PRESL.) STEUD. — This species is endemic to the eastern part of the Pacific coast from the Aleutans to central and southern California. The author's material originates partly from Point Reyes, Marin Co., California, and partly from Huckleberry Hills, one mile S of Monterey, Monterey Peninsula, California. All plants studied are tetraploids with $2n=28$ and sexual with regular meiosis on the male as well as on the female side. It has been observed that the pollen does not develop normally if the plant is exposed to too dry a climate or to cold nights during the growing season. In one such case the Pmc's degenerated and similarities to some of the abnormalities occurring in apomictic species were observed.

Calamagrostis ophitidis (TH. HOWELL), *nov. comb.* — *C. ophitidis* is an endemic species with a very restricted distribution area around the San Francisco Bay and the counties north of the Bay region. Material has been studied from the Tiberon Peninsula, Marin Co., from Rawson Grade, near Occidental, Sonoma Co., as well as from Rutherford, close to Monticelli and Steel Canyon, both in Napa Co. The species



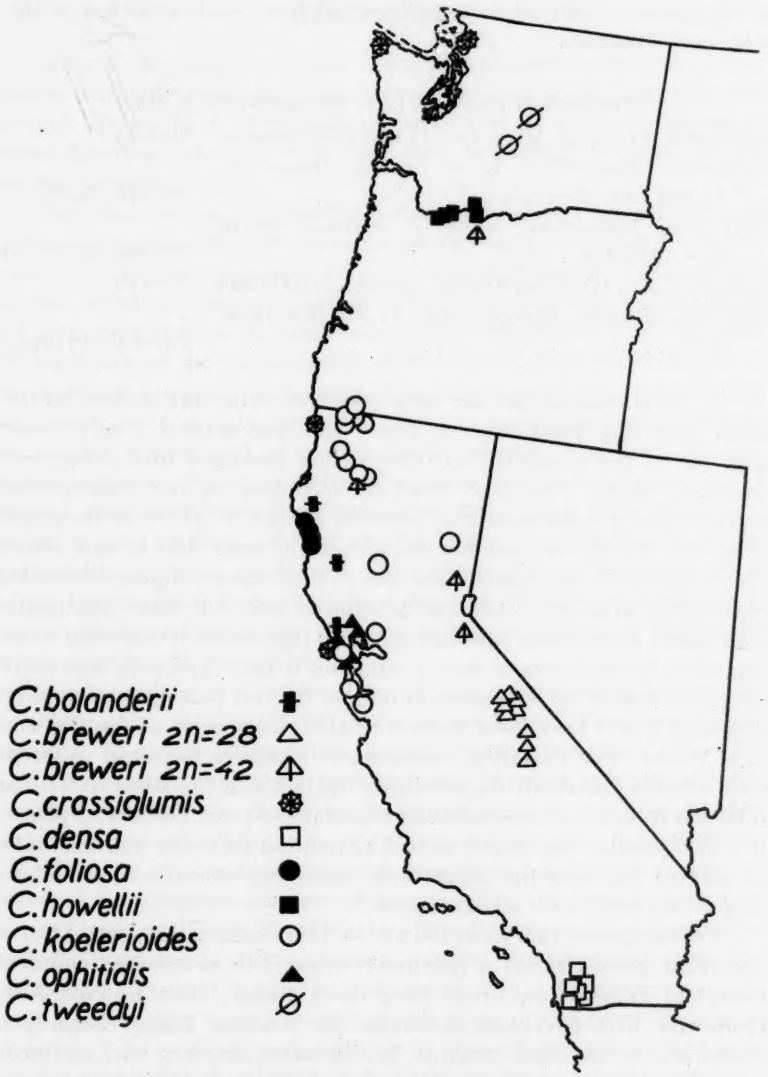
85—87, *C. ophitidis*. 85, M I, 14 II; 86—87, M I in the Emc, 14 II. — 88—92, *C. purpurascens*, 88, plasmodium formed by several fused Pmc's; 89, Emc with one nucleus; 90, the mitotic division in the Emc; 91, Emc with two nuclei; 92, a nucellus with two ES's, probably one diplosporous and one reduced one. — 93, *C. perplexa*. Pollen plasmodium formed by several fused Pmc's.

has also been found on Mt. Tamalpais, Marin Co., N of San Francisco. Several hundred plants have been raised from seeds collected in the localities mentioned; all plants counted are strictly tetraploid with the number $2n=28$. This is of particular interest because of the fact that *C. purpurascens*, which is the closest relative to *ophitidis*, has higher chromosome numbers than this species. *C. ophitidis* is a sexual species with regular meiosis on the male as well as on the female side (Figs. 85—87). Its pollen formation is excellent. The ES is formed according to the normal scheme.

Calamagrostis perplexa SCRIBN. — This relative to *C. porteri* is endemic to Thatcher's Pinnacle, near Ithaca, New York. It is a polyploid species with the chromosome number $2n=70$. In the Pmc's a restricted number of univalents are formed at M I (Figs. 80—82), the average in 173 cells was calculated to $2{,}67 \pm 0{,}18$ per cell, while the number at first anaphase is considerably lower, the mean of 55 anaphase cells being $0{,}62 \pm 0{,}21$ units per cell. At first telophase the number of units has decreased to $0{,}17 \pm 0{,}14$ elements per cell as an average of 50 Pmc's. In some loculi big plasmodia are formed of the same type as those characteristic of apomictic species (Figs. 83 and 93). In the Emc's a regular meiosis occurs with mainly bivalents (Fig. 84); later on an ES is formed according to the normal scheme. Thus, *C. perplexa* is a high-polyploid, sexual species.

Calamagrostis porteri A. GRAY. — Of this eastern North American species the author has obtained material from State College, Penn., collected by Dr. WESTERFELD. As the material was obtained in August, 1953, no panicles have been developed as yet, but the chromosome number has been determined in two plants, which have the numbers $2n=84$ and $2n=87$, respectively. Thus, *porteri* is a high-polyploid just as *perplexa* and it will be of interest to find out if it is sexual, just as the latter species.

Calamagrostis purpurascens R. BR. — *C. purpurascens* has a very wide area of distribution in the northern hemisphere. In Asia it occurs from Hondshu in the South and Lena and Olenek rivers in the West to Chukch Peninsula in the East, it grows on the Commander Islands and is distributed from Alaska and Yukon in the North to California in the South in western North America, whereas in central and eastern North America it is native to Colorado, S. Dakota and Quebec, and finally it is found on Baffin Land and in Greenland. The author has obtained material from California, Colorado and Canada. The chromosome numbers



94. Map of the distribution of the endemic *Calamagrostis* species native to western U. S. A. *C. koelerioides* also occurs outside California and Oregon. *C. tweedyi* is even known to grow in the mountains of northern Idaho.

of the plants, which all have been raised from seeds collected in the wild, are as follows:

Chromosome numbers in C. purpurascens R. Br.

California, Mt. Dana, Mono Co., 11.000 feet elevation	$2n=56$
California, Timberline, E of Coness, Mono Co., 11.000 feet elevation	$2n=48, 49, 53$
California, Timberline, below E Plateau, 10.700 feet elevation	$2n=40, 41, 47, 50$
Canada, North West Territories, Grand Lac de l'Oors	$2n=42$
Colorado, Boulder, Hoosier Pass, 11.000 feet elevation	$2n=54$—57 (9 pl.)

C. purpurascens has not been found to form any pollen. In the loculi only big plasmodia of fused and degenerated Pmc's occur (Fig. 88) of the same type as those earlier described in *C. chalybaea* (Nygren, 1946). The Emc develops according to the diplosporous mitotic scheme characteristic of all other apomictic species of the genus (Figs. 89—92). In one nucellus two ES's were found close to each other. The author is of the opinion that one of these sacs is diplosporous and unreduced, while the other one is reduced and will later degenerate. Two Emc's in the same nucellus now and then occur in different *Calamagrostis* species, even if such a situation is rare. Apospory has never been observed in *Calamagrostis* in spite of the fact that up to now many thousand Emc's have been studied in different species of the Old and New World. The deviating chromosome numbers found in different seed offspring probably depend upon the fact that the seeds have been collected from natural populations containing a great number of plants. It is also possible that pollen as well as reduced ES's now and then may be formed and that the plants with aneuploid chromosome numbers may be the result of a sexual process.

Calamagrostis rubescens Buckl. — This western and central North American species forms a polyploid series. The author has collected tetraploid material on Kings Mts., Coast Range, close to Palo Alto, California, and hexaploid specimens at Wooden Valley, Napa Co., California, at the same time as Dr. Stebbins Jr., has sent me seeds from Huckleberry Hills, near Monterey, California, which have grown up to octoploid plants with $2n=56$. The tetraploid type is sexual and has good pollen formation, while no data as yet are available for the hexaploid and octoploid types.

DISCUSSION.

Two of the North American *Calamagrostis* species studied, *C. bolanderi* and *nutkaensis*, have an isolated position in the genus in this part of the world. *C. bolanderi* probably has its closest relatives in South America, whereas *nutkaensis* may be related to some of the species in eastern Asia.

The three species *C. howellii*, *foliosa* and *tweedyi* are all endemic to the western part of North America, where they grow in isolated populations in very restricted areas. In spite of the fact that the chromosome number of *tweedyi* has not been determined, it is very probable that it is a tetraploid species. It forms morphologically good pollen and does not show any of the characteristic disturbances which are common to the apomictic species of the genus. The three species are probably the last remnants of an old population, which in earlier geological periods has covered a good deal of western North America. The three populations have been adapted to different climates and, therefore, the selection has gone in different directions in the three cases. Each one of the species is very uniform all over its distribution area and each one is ecologically extremely specialized. Thus, the species no doubt are more or less homozygous, a condition in a genus of strongly cross breeding species which will probably soon cause the extinction of these last remnants of an old population.

The conditions in *C. breweri* are of particular interest because of the chromosome races occurring in the species have different ecological demands. The circumstance that the low chromosomal race grows in the high mountains and the type with higher number at lower altitude is not too uncommon in the Arctic. In Scandinavia, for instance, diploid *Anthoxanthum odoratum* grows in the mountains, while the tetraploid is common in the lowland. Diploid *Vaccinium uliginosum* is also a species of the northernmost part of the peninsula, the tetraploid, on the contrary, occurs all over Scandinavia. Diploid *Campanula rotundifolia*, finally, is common in the mountains, whereas the tetraploid grows in the lowland. Thus, it is easy to understand why a hexaploid *breweri* which has its origin from a tetraploid will grow at lower elevation than the latter. It is, however, very difficult to prove that the hexaploid is a direct derivative of the tetraploid, but it must be considered very unlikely that any of the other *Calamagrostis* species now occurring in North America has taken part in the formation of the hexaploid.

Although the tetraploids *C. cainii* and *montanensis* no doubt are

related to each other their populations are of very different sizes. *Cainii* only occurs on a single mountain summit in Tennessee, while *montanensis* is found in seven central states of the U. S. A. and some of the Canadian provinces. Thus, *cainii*, which has a limited ecological amplitude and is morphologically very uniform, belongs to those more or less homozygous species which will soon be extinct, whereas *montanensis* will have better chances to meet changes in the climatical conditions.

The two species *C. ophitidis* and *purpurascens* are closely related to each other, but differ in the way of reproduction. *Ophitidis* is very specialized ecologically and grows only on exposed serpentine rocks, the cracks of which give a poor hold to the roots of the plant. In the restricted area where it is endemic the climate is very uniform year after year, and the species has been adapted to very hot days and cool nights. Morphologically *ophitidis* is just as uniform as ecologically and thus no doubt belongs to the expiring species. In some way this species or its predecessors have entered a diplosporous way of reproduction and given rise to *C. purpurascens*. This development very likely has happened in the same way as in *C. canadensis* and *inexpansa*. *C. purpurascens* varies in different parts of its great area of distribution. Thus, HULTÉN has described a particular subspecies *arctica* characteristic of the northwestern part of the distribution area of the species (HULTÉN, 1941—50, p. 170). The length of the awns varies considerably in different populations, they are particularly long in some populations from the Cascades in the State of Washington. The evolution has entered different ways during the development of the *purpurascens* complex now existing, and in spite of the fact that the species can not be considered a successful apomict which fills its area of distribution with new forms, at least some biotypes of the species may be able to survive if the climate changes.

The two species *C. densa* and *koelerioides* form another pair of closely related species. *Densa* is ecologically and geographically very specialized, morphologically it is uniform, while *koelerioides* has a wider distribution (occurs in five of the western states in the U. S. A.) and even ecologically shows greater variation than *densa*. Thus, *densa* is the expiring and *koelerioides* the successful species of the pair.

In the group made up by the three species *C. rubescens*, *perplexa* and *porteri* the first mentioned one occurs in eight states of western and central U. S. A., while *porteri* is native to four and *perplexa* to one state of the East. *Rubescens* has three chromosome races which to some

extent have different ecological demands, *perplexa*, on the other hand, is a monotypic species which only occurs in one single locality, whereas *porteri* mainly relies on its vegetative propagation by stolons and very rarely flowers. In spite of the fact that we here have three polyploid species to deal with only one of them, *rubescens*, is to be considered successful in the struggle for life.

The species series made up by *C. neglecta*, *inexpansa*, *californica*, *crassiglumis*, and *lapponica* is of particular interest for many reasons. The main differences between *neglecta* and *inexpansa* are so diffuse on the tetraploid level that the two species most likely are to be regarded as different branches of development of the same main type. While *neglecta*, which is the type characteristic of the Old World, has remained tetraploid, *inexpensa* has developed polyploid forms, one of which, *lapponica*, has spread all over the Arctic from Greenland and northern North America to northern Siberia and Europe. Transitional forms with $2n=42$ have been found in Greenland (BÖCHER and LARSEN, 1950, p. 5) and northern Sweden (NYGREN, 1946). Parallel with this production of polyploid forms we meet a tendency of a development against diplospory. The series of different stages covering the area between sexuality and diplospory in *inexpansa* accounted in this paper, make it quite clear that some strains with a tendency to form restitution nuclei on the female side constitute transitional stages between pure sexual types and diplosporous strains. The present author supposed in 1946 that *C. inexpansa* was a sexual in the Rocky Mountains and an apomict in the Aleutans. Now it is obvious that apomictic forms even occur on the North American continent and that *inexpansa* fills the gap between the tetraploid, sexual *neglecta*, the highly polyploid, apomictic *lapponica* and the derivatives of the latter growing in northern Asia. The less successful species in the group no doubt is *C. crassiglumis*, which suffers a languishing existence in some localities along the Pacific coast. Whether it is an apomictic or a sexual species, the gene combination of *crassiglumis* has led to some kind of a blind alley in spite of the very high chromosome number.

The most successful *Calamagrostis* species of the New World no doubt is *C. canadensis*. Just as in *inexpensa* sexual, semiapomictic and more or less obligate apomictic strains occur in this species. This gives *canadensis* a capacity of plasticity which no doubt explains why it is so well prepared for the struggle for life. In comparison with *C. purpurea*, *canadensis* is less variable which may depend upon the fact that it does not intercross so often with other *Calamagrostis* species. *C. pur-*

purea sometimes forms hybrids with *C. canescens* and *neglecta* and now and then with *arundinacea*, while *canadensis* does not seem to cross with any other species of the genus with certainty. *C. scribneri* BEAL might be the combination *C. canadensis*×*rubescens*, however.

The North American *Calamagrostes* give a good idea how the conditions might have been in Europe before the last glaciation. If the North American mountain ranges had run from East to West instead of from North to South, we would probably have had only the following *Calamagrostis* species left in modern time: *C. canadensis, inexpensa, purpurascens*, and *rubescens*. There is a possibility that even *montanensis* and *nutkaensis* would have survived. With this background we might consider old Europe with its ten species as rich in *Calamagrostis*. Three of the ten European species are restricted to smaller areas of distribution, but only one, *C. chalybaea*, is endemic.

SUMMARY.

(1) The chromosome numbers of 19 species of *Calamagrostis* native to North America have been given. The formation of pollen and embryo-sacs has been studied in most of these species.

(2) The chromosome numbers found are the following: *C. bolanderi*, $2n=56$; *C. breweri*, $2n=28$, 42; *C. cainii*, $2n=28$; *C. canadensis*, $2n=42$—66; *C. crassiglumis*, $2n=140$; *C. densa*, $2n=28$; *C. fernaldii*, $2n=84$; *C. foliosa*, $2n=28$; *C. howellii*, $2n=28$; *C. inexpansa*, $2n=28$, 56, 58, 84—104; *C. koelerioides*, $2n=28$, 30; *C. montanensis*, $2n=28$; *C. nutkaensis*, $2n=28$; *C. ophitidis*, $2n=28$; *C. perplexa*, $2n=70$; *C. porteri*, $2n=84$, 87; *C. purpurascens*, $2n=42$—56; and *C. rubescens*, $2n=56$.

(3) The species *C. canadensis* and *inexpansa* have sexual, semi-apomictic as well as apomictic strains. *C. purpurascens* has apomictic strains.

(4) The North American *Calamagrostis* species with apomixis are diplosporous with mitotic division.

(5) Most North American species of the genus are restricted to small areas with a low number of plants in the populations, they are no doubt expiring species. The most successful species are: *C. canadensis, inexpansa, purpurascens*, and *rubescens*. The three first mentioned ones of these species form apomictic strains.

The author is indebted to Dr. LEDYARD STEBBINS, JR., Davis, California, for valuable suggestions and help during several successful excursions in the summer of 1951.

Financial support has been obtained from »Statens naturvetenskapliga forskningsråd».

LITERATURE CITED.

1. BÖCHER, T. W. and LARSEN, K. 1950. Chromosome numbers of some Arctic or Boreal flowering plants. — Medd. om Grønland, Bd. 147, Nr. 6, 32 pp.
2. HULTÉN, E. 1941—1950. Flora of Alaska and Yukon. — Lunds Univ. Årsskr., N. F., Avd. 2, 1902 pp.
3. NYGREN, A. 1946. The genesis of some Scandinavian species of *Calamagrostis*. — Hereditas XXXII, pp. 131—262.

CONTENTS.

Calamagrostis bolanderi THURB. 377
C. breweri THURB. 377
C. cainii HITCHC. 381
C. californica KEARNEY 381
C. canadensis (MICHX.) BEAUV. 381
C. crassiglumis THURB. 383
C. densa VASEY 383
C. fernaldii LOUIS-MARIE 383
C. foliosa KEARNEY 384
C. howellii VASEY 384
C. inexpansa A. GRAY 384
C. koelerioides VASEY 387
C. montanensis SCRIBN. 388
C. nutkaensis (PRESL.) STEUD. 388
C. ophitidis (TH. HOWELL), *nov. comb.* 388
C. perplexa SCRIBN. 390
C. porteri A. GRAY 390
C. purpurascens R. BR. 390
C. rubescens BUCKL. 392
Discussion 393
Summary 396
Literature cited 397

CROSSES WITHIN THE CAMPESTRIS GROUP OF THE GENUS BRASSICA

BY *GÖSTA OLSSON*
SVALÖF, SWEDEN

WITHIN the genus *Brassica* there are at least a couple of wild species and several cultivated ones which have the somatic chromosome number $2n=20$. In many cases the different types are considered to be different species, other authors arrange some of them as subspecies within other 20-chromosome species or even within species with a quite different chromosome number. Finally, some authors consider that the whole group forms one single species.

In order to obtain information regarding the relationships between the different types crosses have been made between available representatives and the fertility and general development of the hybrids have been studied. In the following the different »species» will be denoted with the name under which they have been obtained, and the taxonomical relations will not be discussed until the end of the paper.

MATERIAL AND METHODS.

In the crosses made in 1949 and 1950 the following material has been studied:

Brassica campestris L. var.	*oleifera* METZG.	3 varieties
» » »	*toria* (DUTHIE et FULLER)	1 variety
» » »	*sarson* (yellow seed) PRAIN	2 varieties
» *chinensis* L.		3 »
» *pekinensis* RUPR.		2 »
» *narinosa* BAILEY		2 »
» *nipposinica* BAILEY		1 variety
» *Tournefortii* GOUAN		1 »

The commercial varieties of *oleifera* used (Mette's, Hunsrücker and Fenno spring turnip rape) are used in the breeding work at Svalöf and are cultivated in western Europe. *Toria*, *sarson* and *Tournefortii* have been obtained from Dr. PARTHASARATHY, Indian Agricultural Research Institute, New Delhi, while the material of *chinensis*, *pekinensis*, *nari-*

nosa, and *nipposinica* has been obtained from Professor MIZUSHIMA, Sendai, Japan and Professor NISHIYAMA, Kyoto, Japan. I want to tender these gentlemen my best thanks for their kind helpfulness in sending the seed.

The chromosome number of all the varieties used is $2n=20$, according to KARPECHENKO (1922), SHIMOTOMAI (1925), NAGAI and SASAOKA (1930), ALAM (1936), and SIKKA (1940). The fact has been further confirmed by determinations of the chromosome number of the material here used of *sarson* and *Tournefortii*.

The whole of the *campestris* group — including all the types here used except *Tournefortii* and also turnips and wild *campestris* — is usually characterized by very little waxiness on the rosette leaves and by clasping upper leaves. The inflorescence is usually at first corymbose but gradually becomes racemose. The beak of the pod is well developed and fairly long in comparison with the length of the pod proper. The material is more or less markedly self-sterile (KAKIZAKI, 1922 and 1925; STOUT, 1931; OLSSON, 1953) with the exception of yellow-seeded *sarson*, which is self-fertile (MOHAMMAD, SINGH and ALAM, 1931).

Detailed morphological descriptions of the different types will not be given here, as good descriptions are presented by LUND and KIAERSKOU (1884, 1885), PRAIN (1898), HEGI (1918), SCHULZ (1919, 1936), BAILEY (1922, 1930), SINSKAIA (1928), ALAM (1945), and SUN (1946). On the other hand, a short information of the most important differences between the types in question may be of value for the following discussion.

The wild *Brassica campestris* and turnip rape are morphologically very similar and turnip differs from these mainly by the swollen root. These types occur from Ireland in the west to Japan in the east and from northernmost Norway to Sahara (SINSKAIA, 1928). *Toria* is rather similar to *oleifera* but is less hairy and somewhat more waxy. Yellow-seeded *sarson* is characterized, except by the seed colour and the self-fertility, among other things by the long anthers of the opened flower being introrse. In brown-seeded *sarson*, which unfortunately has not been included in this study, these anthers are extrorse and the plant is self-sterile and, obviously, brown-seeded. *Toria* and *sarson* are important oil plants in northern India and Pakistan.

B. chinensis, *pekinensis*, *narinosa*, and *nipposinica* are used as vegetables in eastern Asia and all have a strongly developed leaf rosette. In *pekinensis* the rosette often forms a head and the leaf stalks are broad and flattened with a notched or jagged serrated wing. In *chinensis*

and *narinosa* the stalks of the lower leaves are thick and white but not winged. The upper leaves of the former are comparatively long and narrow while *narinosa* is characterized by dark green, puckered rosette leaves, broad upper leaves and short and thick pods with a comparatively short and broad beak. The rosette leaves of *nipposinica* are long and narrow, whole or — as in the variety here used — deeply pinnatisect, the pods are comparatively narrow.

The basal leaves of *B. Tournefortii* are hairy and deeply lyrate-pinnatisect and the upper leaves are narrow and not clasping. The flowers are small and pale yellow in colour. The pods are 3,5—6,5 cm in length with a comparatively long beak which usually includes 1—2 seeds. *Tournefortii* is self-fertile and sets seed fairly well when isolated by paper bags. The species occurs as a weed in the Mediterranean region and in the near and middle East as far as the Persian gulf and northern India. It was inadvertently introduced in Australia about the year 1916 and has become a noxious weed on this continent (GARDNER, 1933; CLARKE, 1938; SIMS, 1948); according to MUSIL (1948), it also has appeared in California.

The crosses have been made after artificial emasculation and by artificial pollination; the flowers have been included in pergamyn bags. When crosses are made between well-defined species of *Brassica* some seeds are usually obtained which give rise to plants exactly similar to the mother. Such plants have been obtained in this case in crosses including *B. Tournefortii*. In the other cross combinations typical F_1 plants have been obtained and at any rate the number of maternal progenies has been very low. This is most clearly evident in the cases where the white flowering and yellow-seeded *sarson* has been used as mother. Out of 275 plants grown in 1951 only one was white-flowered and thus matromorphic.

The F_1 material has been grown in six replicates, each plot consisting of one row with 20 plants in 1950 and 10 in 1951. The frequency of well developed and well stained pollen has been determined in a mixture of aceto-carmine and glycerine (1 : 1). With pollen fertility is meant the percentage of well developed pollen grains. The seed fertility is given as the number of well developed seeds in per cent of ovules, as counted in the ripe pods. Root tips have been fixed according to Karpechenko and flower buds with Carnoy. The material fixed has been embedded in the usual manner, cut and stained with crystal violet.

RESULTS.

CROSSES INCLUDING B. TOURNEFORTII GOUAN.

Attempts have been made to cross *B. Tournefortii* with other species with the same chromosome number. As seen from Table 1, no hybrid seed has been obtained but a number of matromorphic plants. Similar results were obtained by MOHAMMAD and SIKKA (1940) in crossing *Tournefortii* with *sarson*. There is, thus, a marked barrier of incompatibility between *Tournefortii* and the other species here studied belonging to the *campestris* group. This barrier is not complete, however, since SIKKA (1940) succeeded to obtain, from 25 pollinated flowers, 2 hybrids between *Tournefortii* and *B. trilocularis* H. f. T. (=*sarson*). These were completely sterile, however, and in diakinesis they usually had 0—3 bivalents and 20—14 univalents; sometimes also one tri- or quadrivalent.

TABLE 1. *Crosses with B. Tournefortii.*

Combination	Number of flowers pollinated	Number of pods developed	Number of hybrid seeds	Number of matromorphic seeds
B. Tournefortii × *B. campestris* var. *oleifera*	239	38	0	127
» » × » » » *toria*	101	15	0	10
» » × » *chinensis*	113	14	0	13
» » × » *pekinensis*	104	12	0	19
» » × » *narinosa*	84	7	0	28
» *chinensis* × *B. Tournefortii*	168	35	0	81
» *narinosa* × » »	157	26	0	45
Total	966	147	0	323

As *B. Tournefortii* is morphologically quite distinct from *B. campestris* s. lat., usually does not form hybrids with this species, and the hybrids, when formed, are sterile, they must be considered as two distinct species. It is therefore misleading to call *B. Tournefortii* »wild turnip» as is often done in papers in the English language (GARDNER, 1933; MUSIL, 1948; GATES, 1950, and others).

CROSSES BETWEEN B. CAMPESTRIS, CHINENSIS, PEKINENSIS, NARINOSA, AND NIPPOSINICA.

The crosses were made in 1949 and 1950 and the hybrids obtained were grown in 1950 and 1951.

In 1949 *chinensis*, *pekinensis*, *narisona*, and *nipposinica* as well as *toria* and *sarson* were crossed mainly with varieties of *B. campestris* grown as summer turnip rape in Sweden or tested as such in the breed-

ing material. As seen from Table 2, a couple of varieties of each »species» were used. In 1950 diallel and reciprocal crosses were made between the species listed in Table 3; *nipposinica* flowered too late, however, to be used as mother.

As seen from Tables 2 and 3, the seed setting is fairly good in all combinations tested. There are some differences between different combinations in regard to the number of seeds per pollinated flower, but the material has flowered and been crossed at different times and usually only 50—60 flowers were pollinated each year for each individual combination; it therefore hardly seems justified to draw any further conclusions than that all the »species» intercross easily and that they are not separated by any barriers to such crosses. In the crosses of 1950 *toria* has set less seed when pollinated with »extra-specific» pollen than the other varieties.

The high degree of »interspecific» crossability obtained in this study is in good agreement with earlier results. Already the work by KAKIZAKI (1925) indicates the interspecific fertility, although his material was very small. SINSKAIA (1927) has shown beyond doubt that the following combinations succeed very easily:

Brassica campestris L. × *B. pekinensis* RUPR., and reciprocally
» » × » *nipposinica* BAILEY
» » × » *chinensis* L., and reciprocally
» *chinensis* L. × » *nipposinica* BAILEY

MORINAGA (1928) crossed *rapa* with *chinensis*, *pekinensis* and »*japonica*»[1] and *chinensis* with *pekinensis* and is of the opinion that different species with $n=10$ are easily intercrossed; the same opinion is expressed by TERASAWA and SHIMOTOMAI (1928) who have made similar crosses. PEARSON (1928) crossed *campestris*, *rapa*, *pekinensis*, and *chinensis* and also found the crosses easy to make; the opinion is further

[1] The name »*Brassica japonica*», usually with SIEB. as author, is used by Japanese writers for material with $2n=20$, which most probably is identical with *Brassica nipposinica* BAILEY (cf. IINUMA and MAKINO, 1912, pl. 33 and BAILEY, 1922, p. 90). The name *japonica* was used, however, already by THUNBERG (*Sinapis japonica*, Flora Jap. 262, 1784) for material which is more related to *Brassica juncea* and according to BAILEY (1922, p. 93) the same material corresponds to *Brassica japonica* SIEB. In these circumstances the specific name *japonica* may not be used as is done by MORINAGA and others, but should in these cases be replaced by *nipposinica* BAILEY. In this paper »*japonica*» (within quotation marks, added by the present author) is used only in quotations from other authors and indicates the same material which is otherwise here denoted as *nipposinica*.

TABLE 2. *Seed setting and pollen fertility in crosses between B. campestris, oleifera, toria, sarson, chinensis, pekinensis, narinosa, and nipposinica, 1949—1950.*

Combination	1949		1950, F_1	
	Number of flowers pollinated	Number of seeds per flower	Number of plants	Pollen fertility
B. campestris v. *oleifera* (Mettes)	—	—	100	95,6
» » » » ×*B. campestris* v. *oleifera* (Mettes×Fenno)	30	11,5	100	93,2
Reciprocally	66	16,2	100	96,1
B. campestris v. *oleifera* (Fenno)	—	—	100	93,0
» *chinensis* (Komatsuna)	—	—	64	95,8
» » » ×*B. campestris* v. *oleifera* (Mettes)	53	11,1	100	95,8
Reciprocally	64	5,5	100	96,1
B. chinensis (Seppaku Taisai)	—	—	100	96,9
» » » » ×*B. campestris* v. *oleifera* (Mettes)	73	10,0	100	95,4
» *chinensis* (Hiroshima)	—	—	42	93,1
» » » ×*B. campestris* v. *oleifera* (Mettes)	61	3,7	100	92,9
» *pekinensis* (Matsushima)	52	0,6	96	91,9
» » » ×*B. campestris* v. *oleifera* (Mettes)	55	4,9	100	96,5
» *pekinensis* (Santasai)	—	—	100	95,3
» » » ×*B. campestris* v. *oleifera* (Mettes)	57	14,9	100	92,3
» *campestris* from Tabor	—	—	100	92,2
» » ×*B. campestris* v. *oleifera* (Fenno)	48	6,3	100	93,7
» *campestris*×*B. pekinensis* (Matsushima)	48	5,9	100	97,3
» *chinensis* (Seppaku Taisai)×*B. pekinensis* (Matsushima)	57	6,3	100	96,3
Reciprocally	51	1,1	22	96,6
B. narinosa from Sendai	—	—	84	94,4
» » ×*B. campestris* v. *oleifera* (Hunsrücker)	57	8,1	100	93,8
Reciprocally	67	2,7	96	95,7
B. campestris v. *oleifera* (Hunsrücker)	—	—	100	91,6
» *narinosa* from Kyoto	—	—	100	91,0
» » ×*B. chinensis* (Seppaku Taisai)	50	13,7	100	94,4
Reciprocally	60	11,8	100	93,6
B. narinosa×*B. pekinensis* (Matsushima)	48	1,8	42	95,0
» *campestris*×*B. nipposinica*	52	5,3	97	96,1

Combination	1949		1950, F_1	
	Number of flowers pollinated	Number of seeds per flower	Number of plants	Pollen fertility
B. nipposinica	—	—	45	94,7
» *campestris* v. *toria*×*B. campestris* v. *oleifera* (Mettes)	63	2,3	49	90,7
» *campestris* v. *sarson* T 74×*B. campestris* v. *oleifera* (Mettes)	56	5,1	35	79,9
» *campestris* v. *sarson* T 87×*B. campestris* v. *oleifera* (Mettes)	56	9,1	48	88,6

supported by BRUNE (1949) who states that »der Kreuzbarkeit nach bildeten *Brassica pekinensis, chinensis* und Teltower Rübe eine enge Gruppe». *Sarson* is easily crossed with *campestris* but only with difficulty with *napus* (SINSKAIA, 1927). MOHAMMAD, SINGH and ALAM (1931) have shown that *toria, sarson* and turnip are easily intercrossed and this has been confirmed concerning the two last named types by SIKKA (1940).

Neither wild *Brassica campestris* nor turnip have been included in the present series of experiments. The results obtained by LUND and KIAERSKOU (1885) as well as the author's own experience from earlier work (unpubl.) show, however, that turnip, turnip rape and wild *campestris* are easily intercrossed, a fact also pointed out by BAUR (1939) and DAVEY (1939). The experience of the present author as well as earlier results by LUND and KIAERSKOU (1885), SINSKAIA (1927) and BAUR (1939) show that spontaneous hybrids between the types discussed in this paper are fairly common.

In most characters the different F_1's are intermediate between the respective parents and no detailed study of the morphology of the

TABLE 3. *Number of hybrid seeds per pollinated flowers in crosses between different 20-chromosome forms of Brassica, 1950.*

♀ \ ♂	*B. campestris* var. *oleifera*	*B. campestris* var. *toria*	*B. campestris* var. *sarson*	*B. chinensis* (Seppaku)	*B. pekinensis* (Santasai)	*B. narinosa*	*B. nipposinica*
B. campestris var. *oleifera* .	*12,1*	13,4	6,0	14,1	16,8	13,4	—
» » » *toria*	5,1	*10,8*	6,4	4,0	4,9	10,0	—
» » » *sarson* ..	14,6	11,2	*14,6*	8,4	4,1	7,0	—
» *chinensis* (Seppaku)	7,9	6,4	6,3	*8,5*	8,6	11,8	3,5
» *pekinensis* (Santasai) ...	19,1	13,9	1,8	10,1	*5,4*	5,0	6,6
» *narinosa*	8,9	4,8	4,9	11,5	5,6	*5,1*	9,0

TABLE 4. *Pollen fertility in F_1 of crosses between different 20-chromosome forms of Brassica, 1951.*

♀ \ ♂	*B. campestris* var. *oleifera*	*B. campestris* var. *toria*	*B. campestris* var. *sarson*	*B. chinensis* (Seppaku)	*B. pekinensis* (Santasai)	*B. narinosa*	*B. nipposinica*
B. campestris var. *oleifera* .	*95*	95	78	97	97	96	—
» » » *toria*	96	*95*	80	94	95	92	—
» » » *sarson* ..	77	83	*95*	83	68	75	—
» *chinensis* (Seppaku)	95	94	77	*93*	97	97	94
» *pekinensis* (Santasai) ...	96	86	82	95	*93*	80	96
» *narinosa*	97	92	73	94	96	*95*	94

hybrids has been made. A couple of characters, however, are evidently recessive. The yellowish white flower colour and the yellow seed colour of the type of *sarson* here used are both evidently caused by genes which are recessive to the genes which in all the other forms used cause yellow flower colour and brown seed. MOHAMMAD and SIKKA (1937) have shown that yellow seed colour in *sarson* is recessive to brown seed colour in *toria* and *sarson*.

The pollen fertility in F_1 is given in Tables 2 and 4 and the seed fertility in Table 5. The fertility percentages given in Tables 4 and 5 are the means of values obtained from 50—60 plants. As seen from the tables, crosses including *B. campestris* var. *sarson* have practically always a lower fertility than the parent species, while the other hybrids — with a few exceptions — have the same fertility as the parents. There

TABLE 5. *Seed setting in F_1 of crosses between different 20-chromosome forms of Brassica, 1951.*

♀ \ ♂	*B. campestris* var. *oleifera*	*B. campestris* var. *toria*	*B. campestris* var. *sarson*	*B. chinensis* (Seppaku)	*B. pekinensis* (Santasai)	*B. narinosa*	*B. nipposinica*
B. campestris var. *oleifera* .	*78*	78	54	83	83	81	—
» » » *toria*	79	*78*	59	86	78	72	—
» » » *sarson* ..	50	54	*89*	78	65	56	—
» *chinensis* (Seppaku)	80	77	82	77	79	89	86
» *pekinensis* (Santasai) ...	76	77	70	83	75	74	74
» *narinosa*	83	63	54	88	85	*86*	88

is, thus, a comparatively distinct sterility barrier between the yellow-seeded *sarson* and the other types used in this study. Among the F_1's with high fertility there are two combinations (*pekinensis*×*toria* and *pekinensis*×*narinosa*) which have a comparatively low pollen fertility and one combination (*narinosa*×*toria*) with a low seed fertility. The reciprocal combinations are fully fertile, however; furthermore, the two combinations with reduced pollen fertility have a normal seed fertility and the combination which has reduced seed fertility has a normal pollen fertility. It seems most probable, therefore, that the disturbances in the fertility are quite accidental. The time at which the pollen was sampled may be of some importance. The seed fertility is remarkably high in the cross between *chinensis* and *sarson*.

The crosses made by SINSKAIA (1927), MORINAGA (1928), PEARSON (1928), TERASAWA and SHIMOTOMAI (1928), and SIKKA (1940) have also resulted in fully fertile F_1 hybrids.

It has been impossible to grow each F_1 combination in an isolated plot, and it has therefore been impossible to make an adequate study of the fertility in F_2. Seed from the F_1 plots — grown side by side — has been sown, however, and the F_2 material has been studied to some extent, although no detailed determinations of the fertility have been made. The F_2's were vegetatively well developed and showed evident segregation in morphological characters; at the same time it was evident that intercrossing between different F_1's had occurred. There did not occur any marked sterility in F_2 and the seed setting was good. Similar results have been reported by PEARSON (1928) while SINSKAIA (1927) observed a certain reduction of the fertility in F_2 of the crosses *campestris*×*chinensis* and *campestris*×*pekinensis*.

TABLE 6. *Plant height in cm in F_1 of crosses between different 20-chromosome forms of Brassica, 1951.*

♀ \ ♂	*B. campestris* var. *oleifera*	*B. campestris* var. *toria*	*B. campestris* var. *sarson*	*B. chinensis* (Seppaku)	*B. pekinensis* (Santasai)	*B. narinosa*
B. campestris var. *oleifera*	*109*	105	115	128	141	131
» » » *toria*	106	*96*	115	128	127	118
» » » *sarson*	117	111	*99*	141	136	128
» *chinensis* (Seppaku)	142	127	132	*139*	147	143
» *pekinensis* (Santasai)	137	141	143	142	*150*	138
» *narinosa*	132	125	138	146	137	*124*

TABLE 7. *Seed yield in grams per plant in F_1 of crosses between different 20-chromosome forms of Brassica, 1951.*

♀ \ ♂	*B. campestris* var. *oleifera*	*B. campestris* var. *toria*	*B. campestris* var. *sarson*	*B. chinensis* (Seppaku)	*B. pekinensis* (Santasai)	*B. narinosa*
B. campestris var. *oleifera*	*10,3*	12,9	10,8	12,4	14,7	14,2
» » » *toria*	12,9	*8,4*	9,3	13,6	11,1	14,4
» » » *sarson*	11,5	7,6	*1,9*	14,8	15,0	9,1
» *chinensis* (Seppaku)	12,0	13,9	13,0	*5,5*	8,9	5,6
» *pekinensis* (Santasai)	17,1	9,2	15,8	—[1]	—[1]	—[1]
» *narinosa*	14,8	12,2	8,6	7,1	5,1	*5,3*

[1] The values are missing because the late ripening plants were severely damaged by birds.

In order to obtain some estimate of the development of the plants the plant height, weight of seed per plant, number of well-developed seeds per pod and weight of 1.000 seeds have been determined in the material of 1951 and plant height as well as seed weight per plant also in some of the material of 1950. The results of the measurements on the material of 1951 are listed in Tables 6—9. It has been impossible to obtain stands quite without missing plants; furthermore, the number of plants in each plot is fairly small. The data are, thus, not sufficient for a more thorough study of heterosis; nor has such a study been the main aim of the present investigation. However, the data obtained show that the F_1 plants have been vegetatively well developed and have given a good

TABLE 8. *Number of well developed seeds per pod in F_1 of crosses between different 20-chromosome forms of Brassica, 1951.*

♀ \ ♂	*B. campestris* var. *oleifera*	*B. campestris* var. *toria*	*B. campestris* var. *sarson*	*B. chinensis* (Seppaku)	*B. pekinensis* (Santasai)	*B. narinosa*	*B. nipposinica*
B. campestris var. *oleifera* .	*20,9*	23,2	16,5	24,6	25,1	25,0	—
» » » *toria*	24,6	*23,5*	17,9	27,3	23,9	22,2	—
» » » *sarson* ..	14,5	16,1	*33,8*	25,2	21,2	20,7	—
» *chinensis* (Seppaku)	24,9	24,1	26,1	*21,0*	24,2	25,4	28,2
» *pekinensis* (Santasai) ...	25,2	26,0	24,9	26,7	*18,2*	20,6	25,3
» *narinosa*	25,7	18,8	15,2	25,9	23,0	*22,5*	30,8

TABLE 9. *Weight of 1.000 seeds in grams in F_1 of crosses between different 20-chromosome forms of Brassica, 1951.*

♀ \ ♂	*B. campestris* var. *oleifera*	*B. campestris* var. *toria*	*B. campestris* var. *sarson*	*B. chinensis* (Seppaku)	*B. pekinensis* (Santasai)	*B. narinosa*	*B. nipposinica*
B. campestris var. *oleifera*	2,6	2,8	3,6	2,3	2,9	2,5	—
» » » *toria*	3,0	2,7	4,0	2,7	3,1	3,0	—
» » » *sarson*	3,6	4,1	3,0	3,3	4,4	3,5	—
» *chinensis* (Seppaku)	2,3	2,7	3,2	3,0	3,2	2,6	2,9
» *pekinensis* (Santasai)	2,6	2,9	3,8	3,0	3,5	3,1	3,3
» *narinosa*	2,3	3,0	3,5	2,7	3,1	2,7	2,5

yield of seed. If heterosis is defined as superiority over the parent with the highest value (*cf.* HAGBERG, 1952) it has been possible to demonstrate significantly the occurrence of heterosis only in a few cross combinations, but an indication is apparent in many cases. As an example the fact may be mentioned that the yield of seed per plant in all crosses including *oleifera* has been larger in F_1 than in the *oleifera* parent, which latter obviously is the highest yielding of the strains used. As seen from Table 7, the seed yield of F_1 is higher than that of the parents in practically all other combinations also. In this connection it must be kept in mind that these parents are adapted to environments quite different from the one in which they have here been tested and that some of them must have been selected with regard to their value as vegetables with small consideration, probably, to their yield of seed.

If the partially sterile hybrids with *sarson* are excepted the number of well developed seed per pod is larger in the F_1's including *oleifera* than in this »species», and in this character evident heterosis — superiority to the best parent — occurs in many cases.

In combinations with *sarson* the size of the seed is larger than in the parents. To some extent this may be due to the partial sterility with the resulting low number of seeds per pod, but this can not be the only cause since the weight of 1.000 seeds is high also in the combinations between *sarson* and *chinensis* or *pekinensis* where the pods have a normal number of seeds.

As already mentioned, only part of the F_1 material grown in 1950 was thrashed and determinations made of plant height and seed yield per plant. The results obtained in this limited material agree with those

TABLE 10. *Cytological observations at first metaphase.*

Material	PMC's at M_I having the number of bodies in polar view			the number of univalents		
	9	10	11	0	1	2
B. campestris var. *oleifera*	—	98	2	99	1	—
» » » *sarson*	1	96	1	125	—	—
» » varr. *oleifera*×*sarson* ...	3	115	4	177	10	4
» » » *sarson*×*oleifera* ...	—	97	1	178	5	—
» » » *oleifera*×*toria*	—	50	—	80	—	—
» » » *toria*×*sarson*	1	71	3	108	3	—
» *chinensis*×*B. campestris* var. *sarson*	1	85	4	98	2	—

from the material of 1951. Among the data from the material of 1950 may be pointed out the fact that while no heterosis in regard to seed yield was observed in F_1 between two commercial varieties of *oleifera* (Mette's and Fenno) it was more or less marked in the combination *oleifera*×*narinosa* and *oleifera*×*chinensis*. In the first mentioned of these combinations the *t*-value for (F_1—P_{max}) was $2{,}34^*$ and in the latter it was $4{,}54^{***}$ (*cf.* BONNIER and TEDIN, 1940).

The vigorous development and the high yield of seed in the F_1 material here studied indicate that in the breeding of turnip rape it is not necessary to limit the material to the *oleifera* material grown in north-western Europe but that it is possible to utilize the variation within the entire *campestris* group.

In regard to the cytology of the hybrids PEARSON (1928) states, »although no detailed cytological investigation has been made of these crosses, the preliminary study indicates that pairing is normal and that no irregularities occur at the reduction division». MORINAGA (1928) states that the reduction division is normal in the hybrids studied by him and the same holds true of the hybrid between *pekinensis* and »*japonica*»

TABLE 11. *Number of chromosomes at second metaphase.*

Material	Number of chromosomes in second metaphase plates		
	9	10	11
B. campestris var. *oleifera*	—	60	1
» » » *sarson*	1	76	1
» » varr. *oleifera*×*sarson*	3	97	—
» » » *sarson*×*oleifera*	2	56	—
» » » *oleifera*×*toria*	—	100	—
» » » *toria*×*sarson*	—	50	—
» *chinensis*×*B. campestris* var. *sarson*	7	56	—

investigated by TERASAWA and SHIMOTOMAI (1928). In the F_1 hybrids *rapa*×*chinensis* and *chinensis*×*pekinensis* RICHHARIA (1937) found regular meiosis, while in the combination *pekinensis*×*rapa* »occasionally two free univalents were observed and occasionally separation was into 9 and 11 instead of 10 and 10». In the hybrid between *trilocularis* (=*sarson*) and *rapa* SIKKA (1940) observed complete pairing in diakinesis and first metaphase while non-disjunction occurred in some instances at first anaphase.

Because of the results just mentioned and the general good fertility it was not considered necessary to carry out a detailed cytological analysis of the present material; such analysis was limited mainly to some crosses including *sarson*. The chromosomes are comparatively small and the study has been limited to a determination of the number of bodies in polar views of the first metaphase and the number of univalents outside the plate and to the number of chromosomes at second metaphase. As seen from Tables 10 and 11, the hybrid *oleifera*×*toria* has a completely regular meiosis. Even the combinations with *sarson* which have been studied have on the whole a regular meiosis but the frequency of univalents is somewhat higher than in the parents and the chromosome pairing thus evidently somewhat weaker. There is a very considerable difference in this respect between different F_1 plants.

DISCUSSION.

As mentioned in the introduction, the »species» here studied are taxonomically grouped in widely different way by different authors. As an example may be mentioned that PRAIN (1898) considers *B. chinensis* L. as a variety of *B. oleracea* L., while SCHULZ (1919, 1936) includes it in *B. napus* L. as a separate variety. With good reasons SINSKAIA (1928) criticizes SCHULZ's grouping and considers *chinensis* as a separate species, closely related to *campestris*. The criticism of SINSKAIA gains very much in strength from the elucidation of the relationships between the *Brassica* species which is a result of the genome analysis of MORINAGA (1929 a, 1934) and from the synthesis by U (1935) of rape. These investigations have shown that *B. napus* ($n=19$) is an allopolyploid, a combination between genomes from the *oleracea* group ($n=9$) and the *campestris* group ($n=10$). SCHULZ's grouping of *chinensis* together with *napus* is thus definitely erroneous.

PRAIN mistakenly believes *toria* to be a variety of *napus* [»Toria seems to be the same plant as *B. praecox* (summer rape), or if not the

same is at least very like and very near it»; PRAIN, 1898, p. 78]. PRAIN's classification is accepted by SCHULZ (1919, 1936), who includes *toria* as well as *sarson* in *napus*. In several papers by MOHAMMAD and co-workers (1931, 1935, 1937 and 1940) *toria* is given the name *B. napus* L. var. *dichotoma* PRAIN. Other taxonomists (see ALAM, 1945) give *toria* the astounding combination *B. campestris* ssp. *napus*. ALAM (*l.c.*) follows this system, adding a varietal name, so that *toria* becomes *B. campestris* L. ssp. *napus* var. *toria* D. et F. On the other hand, HOWARD (1940) is of the opinion that the name *napus* should be restricted to forms with $2n=38$. This seems a reasonable limitation and there is no reason whatever for including *napus* in the latin name of *toria*. The correct name for this type should be *B. campestris* L. ssp. *dichotoma* (ROXB.) OLSSON.

The question whether all the types here discussed are to be considered as definite species or are to be combined in one or a few specific groups depends largely upon the way in which the species concept is defined. If the definition of BAILEY (1896, p. 457) is accepted, that a species is »the unit in classification, designating an assemblage of organisms which, in the judgment of any writer, is so marked and so homogeneous that it can be conveniently spoken of as one thing» all the types here treated should be treated as separate species. Quite a different species concept is advocated by other authors, *e. g.* HERIBERT NILSSON (1930), MÜNTZING, TEDIN and TURESSON (1931), DOBZHANSKY (1935, 1941), CLAUSEN (1936), CLAUSEN, KECK and HIESEY (1939), MAYER (1940, 1942), and STEBBINS (1950). STEBBINS says, »All agree that species must consist of systems of populations that are separated from each other by complete or at least sharp discontinuities in the variation patterns, and that these discontinuities must have a genetic basis. That is, they must reflect the existence of isolating mechanisms which greatly hinder or completely prevent the transfer of genes from one system of populations to another» (page 34). In spite of the difficulties met with in applying these principles to cultivated species or groups of species (*cf.* MAC KEY, 1954) it is desirable, in the author's opinion, to do it as far as possible since a taxonomical system founded on these principles gives the best picture possible of the interrelationships between the different »species» and other units. In *Brassica*, furthermore, systematic breeding has so far not to any great extent veiled the grouping which has been developed by a more or less natural selection in which the part of Man has mainly been unconscious.

If the different units within the *campestris* group are judged from this point of view it is evident that *B. Tournefortii* GOUAN is to be con-

sidered as a separate species, being morphologically well distinguished from the others and also separated from them by a strong barrier at incompatibility.

Yellow-seeded *sarson* also differs morphologically from all other types within *campestris* s. lat. (*cf.* ALAM, 1945) and is also characterized by self-fertility in contrast to the self-sterility of the other types. It is easy to cross with the other types within the group, but the hybrids usually show a marked tendency to decreased fertility. There are, thus, some facts in favour of the opinion of SINSKAIA (1928) and ALAM (1945) that yellow-seeded *sarson* should be given specific rank. On the other hand, the importance of the reduction in fertility should not be overestimated; it must be kept in mind that reduced fertility may occur also in hybrids within an undoubtedly good Linnean species (MÜNTZING, 1929, 1938). In the opinion of the present author the factor deciding the specific value of yellow-seeded *sarson* must be whether it will remain as a distinct unit type in its natural area of distribution. There are no data available which indicate such an isolation of the type in question. On the other hand, MOHAMMAD, SINGH and ALAM (1931) state that *toria* and *sarson* intercross freely in the fields and that »it was very difficult to keep the various varieties pure». Unfortunately, there is no information whether this statement holds true of brown-seeded or yellow-seeded *sarson* or of both types, but on the other hand it is not restricted only to the brown-seeded type. According to MOHAMMAD (1935), it is possible by crossing to introduce the self-fertility of yellow-seeded *sarson* into brown-seeded types as well as into *toria*, and in regard to this character, to the position of the anthers (extrose—introse) and to the hairiness of the leaves MOHAMMAD and SIKKA (1937) obtained monohybrid segregation in the cross between *sarson* and *toria*. The seed colour, on the other hand, is regulated by several genes. It seems most suitable, at least provisionally, to arrange yellow-seeded *sarson* as a well differentiated subspecies of *B. campestris*. As far as the author has been able to find out, it should be given the latin name *Brassica campestris* L. ssp. *trilocularis* (ROXB.) OLSSON (*cf.* ALAM 1945, p. 179).

The other types here tested, *viz. oleifera*, *toria*, *chinensis*, *pekinensis*, and *nipposinica*, as well as wild *B. campestris* and turnip, are not separated by any barriers and frequently intercross spontaneously. The hybrids are, with few exceptions, fully fertile and have a normal meiosis. When crossed with *napus* and *juncea* all forms so far tested give $10_{II}+9_{I}$ and $10_{II}+8_{I}$, respectively (MORINAGA, 1929 a and b; SASAOKA, 1930; U, 1935; and others).

According to serological investigations by FUKUSHIMA and MARUYAMA (1929), »*japonica*», *pekinensis*, *rapa*, and *chinensis* (?) form a uniform group, distinct from *napus*, *juncea* and *oleracea*. SHIMOTOMAI (1925) states, »according to the taxonomical view of Mr. MAKINO, *B. campestris*, *B. Pe-tsai* (=*pekinensis*), *B. rapa*, *B. chinensis* and *B.* »*japonica*» are intimately related with each other». PEARSON (1928) is of the opinion that »these species should be reduced to subspecies rank».

Between the opinions offered by BAILEY and PEARSON there are several intermediate ones; SINSKAIA (1927, 1928) and SUN (1946) may be quoted as examples. SUN divides the material with 20 chromosomes in one European species, *B. campestris*, and one East-Asiatic, *B. chinensis*, a grouping which is criticized by GATES (1950) as »an undesirable degree of lumping». SUN evidently does not know the Indian material, nor does he discuss *narinosa*. *Pekinensis* is regarded as a variety of *chinensis*. SUN has studied altogether too few of the existing types to be able to discuss the taxonomy of the whole group.

SINSKAIA, on the other hand, seems to be the one who has studied the largest number of *Brassica* forms and in her papers of 1927 and 1928 she gives very important information about the relations between the different »species». She places *oleifera* and *rapifera* (=*rapa*) as varieties of *campestris* but gives specific value to each of *chinensis*, *pekinensis* and *nipposinica*. The difference between the system of SINSKAIA and the one here presented is fairly small. The important thing seems to be to indicate which taxonomical units are closely related; whether they are given specific rank or not is mainly a question of definition. According to the opinion of the present author, however, the differences between the different types within the *campestris* group are not larger than those betwen different varieties of *B. oleracea* (*e. g.*, cabbage and cauliflower), which are commonly considered to belong all to one species. The difference between, *e. g.*, *chinensis* and *pekinensis* is smaller than that between some of the *oleracea* varieties and not at all comparable with the differences between *oleracea* and rape or between rape and turnip rape. It seems desirable that the different species of *Brassica* should be as equivalent as possible and this is a strong argument in favour of including the whole of the *campestris* group into one species only.

The taxonomical unit next below species is sometimes called varietas, sometimes subspecies. In accordance with the opinions expressed by CLAUSEN (1936), MAYER (1942) and STEBBINS (1950) the term

subspecies is used here. It has been preferred to the term subspecioid suggested by MANSFELD (1953) since both wild and cultivated subspecies are included.

The types here discussed may then be arranged according to the following scheme:

Species *Brassica campestris* L. s. lat.

ssp. *eu-campestris* (L.) OLSSON, ssp. nova. — (*B. campestris* L., Sp. Pl. 666. 1753.) — Wild type.

» *oleifera* (METZG.) SINSK. — SINSKAIA, Bull. Appl. Bot. Plant-breed. 19: 3, p. 56. 1928. — (*B. rapa* ssp. *oleifera* METZGER, Syst. Beschreib. cult. Kohlart 49. 1833.) — Turnip rape.

» *rapifera* (METZG.) SINSK. — SINSKAIA, Bull. Appl. Bot. Plant-breed. 19: 3, p. 103. 1928. — (*B. rapa* L., Sp. Pl. 666. 1753. — *B. rapa* ssp. *rapifera* METZGER, Syst. Beschreib. cult. Kohlart 52. 1833. — *B. campestris* ssp. *rapa* (L.) HOOK. f. et ANDS., Fl. Brit. Ind. I: 156. 1875.) — Turnip.

» *chinensis* (L.) MAKINO, Somoku-Dzusetsu, I: 3, Vol. 12, Tab. XXVII. 1912. — (*B. chinensis* L., Cent. Pl. I, 1755 and Amoen. Acad. IV: 280. 1759. — BAILEY, Gent. Herb. I: 99. 1922.)

» *pekinensis* (LOUR.) OLSSON, comb. nova. — (*Sinapis pekinensis* LOUREIRO, Fl. Cochin. 485. 1793. — *B. pekinensis* RUPRECHT, Fl. Ingr. 96. 1860.) — Pe-tsai.

» *narinosa* (BAILEY) OLSSON, comb. nova. — (*B. narinosa* BAILEY, Gent. Herb. I: 99. 1922.)

» *nipposinica* (BAILEY) OLSSON, comb. nova. — (*B. nipposinica* BAILEY, *l. c.*, 86. 1922.)

» *dichotoma* (ROXB.) OLSSON, comb. nova. — (*Sinapis dichotoma* ROXBURGH, Fl. Ind. III: 117. 1832. — *B. napus* var. *dichotoma* PRAIN, Agric. Ledger I: 36. 1898.) — Toria.

» *trilocularis* (ROXB.) OLSSON, comb. nova. — (*Sinapis trilocularis* ROXBURGH, Fl. Ind. III: 121. 1832. — *B. trilocularis* em. ALAM, Ind. Journ. Agr. Sci. 15: 179. 1945.) — Yellow-seeded sarson.

Species *Brassica Tournefortii* GOUAN, Illustr. Obs. Bot. 44, 1773, Tab. XX A.

It must be pointed out, however, that this list is not complete. *B. campestris* s. lat. should at least include also brown-seeded sarson and *B. parachinensis* BAILEY. PRAIN (1898) combines both types of sarson under the name *B. campestris* var. *sarson*. DUTHIE (1903) com-

bines brown-seeded sarson with toria under the name *B. napus* var. *dichotoma*. ALAM (1945) considers the two latter »as belonging to different subspecies of a single species» and calls brown-seeded sarson *B. campestris* L. subsp. *campestris* var. *dichotoma* WATT. and toria *B. campestris* L. subsp. *napus* var. *toria* D. et F. It must be left to further investigations to decide definitely the relations between toria and the two types of sarson.

B. parachinensis BAILEY is »distinguished from *B. chinensis* in the petiolate and not clasping stem-leaves» (BAILEY, 1930). SINSKAIA (1927) sees it as a variety of *chinensis* and most probably they are closely related. According to SINSKAIA, it is easily crossed with *nipposinica* and the hybrid is fully fertile.

It may also be discussed whether all the types should be placed as equivalent subspecific units. The difference between *eu-campestris* and *oleifera* is rather minute and possibly they ought to be grouped together. It is convenient, however, to distinguish in a simple manner between the wild form and the cultivated one, of which several high-bred commercial varieties exist. Possibly *pekinensis*, *narinosa* and *nipposinica* should be placed as varieties of *chinensis* but such an arrangement does not seem at present to offer any advantages.

The variation within *B. Tournefortii* has not been investigated.

SUMMARY.

Crosses have been made between different *Brassica* forms with the haploid chromosome number $n=10$. The vitality and fertility of the hybrids obtained have been determined and considering the results obtained the taxonomy within the group is discussed. *B. Tournefortii* which usually cannot be crossed with *B. campestris* s. lat. is considered to be a separate species. Wild *B. campestris*, turnip rape, turnip, toria, sarson and different types of »chinese cabbage» are easily intercrossed and the hybrids are fertile, except when yellow-seeded sarson is one of the parents. These types, therefore, are all considered to be subspecies of *B. campestris* s. lat. (see page 414); yellow-seeded sarson (*B. campestris* ssp. *trilocularis*) is somewhat more different from the others than these are *inter se*.

The investigation has been supported by grants from the Sales Association of the Manufacturers of Oleomargarine.

LITERATURE CITED.

1. ALAM, Z. 1936. Cytological studies of some Indian oleiferous *Cruciferae*. III. — Ann. Bot. 50: 85—102.
2. — 1945 Nomenclature of oleiferous *Brassicas* cultivated in the Punjab. — Indian Journ. Agr. Sci. 15: 173—181.
3. BAILEY, L. H. 1896. The philosophy of species-making. — Bot. Gaz. 22: 454—462.
4. — 1922. The cultivated *Brassicas*. — Gentes Herbarum I: 53—108.
5. — 1930. The cultivated *Brassicas*. Second paper. — Gentes Herbarum II: 211—267.
6. BAUR, G. 1939. Raps, *Brassica Napus* L. var. *oleifera* METZGER, Rübsen, *B. Rapa* L. var. *oleifera* METZGER. — In ROEMER-RUDORF, Handbuch der Pflanzenzüchtung IV: 206—242.
7. BONNIER, G. and TEDIN, O. 1940. Biologisk variationsanalys. — Stockholm.
8. BRUNE, W. 1949. Genetische Beobachtungen innerhalb der Gattung *Brassica*. — Zschr. f. Pflanzenzücht. 28: 144—185.
9. CLARKE, G. H. 1938. Important weeds of south Australia: Nos. 23 and 24. Wild turnips (*Brassica Tournefortii*, GOUAN, *Rapistrum rugosa*, ALL.). — South Austral. Dept. Agr. Journ. 41: 804—808.
10. CLAUSEN, J. 1936. The basis for natural systematic units. — Nordiska (19. skandinaviska) naturforskarmötet i Helsingfors: 520—523.
11. CLAUSEN, J., KECK, D. and HIESEY, W. H. 1939. The concept of species based on experiment. — Amer. Journ. Bot. 26: 103—106.
12. DAVEY, V. M. 1939. Hybridization in *Brassicae* and the occasional contamination of seed stocks. — Ann. Appl. Biol. 26: 634—636.
13. DOBZHANSKY, TH. 1935. A critique of the species concept in biology. — Philosophy of Science 2: 344—355.
14. — 1941. Genetics and the origin of species. Second edition. — New York.
15. DUTHIE, J. F. 1903. Flora Upper Gangetic Plain. I: 43—46. — Calcutta.
16. FUKUSHIMA, E. and MARUYAMA, Y. 1929. Preliminary report of the serological examination on *Brassica*. — Proc. Imp. Acad. Tokyo 5: 473—476.
17. GARDNER, C. A. and THROSSELL, G. L. 1933. The wild turnip (*Brassica Tournefortii* GOUAN); with notes on cultural methods of control. — West Austral. Dept. Journ. 10: 409—419.
18. GATES, R. 1950. Genetics and taxonomy of the cultivated *Brassicas* and their wild relatives. — Bull. Torrey Bot. Club 77: 19—28.
19. HAGBERG, A. 1952. Heterosis in F_1 combinations in *Galeopsis*. I and II. — Hereditas XXXVIII: 33—82 and 221—245.
20. HEGI, G. 1918. Illustrierte Flora von Mittel-Europa. IV, 1: 229—263. — München.
21. HOWARD, H. W. 1940. The nomenclature of *Brassica* species. — Curr. Sci. 9: 494—495.
22. IINUMA, Y. and MAKINO, T. 1912. Sōmoku-Dzusetzu; or, An iconography of plants indigenous to, cultivated in, or introduced into Nippon (Japan). Part I: III. Vol. 12: Pl. XXVII—XLI. — Tokyo.
23. KAKIZAKI, Y. 1922. Self-sterility in Chinese cabbage. — Journ. Hered. 13: 374—376.
24. — 1925. A preliminary report of crossing experiments with Cruciferous plants. — Jap. Journ. Genet. III: 49—77.
25. KARPECHENKO, G. D. 1922. The number of chromosomes and the genetic cor-

relation of cultivated *Cruciferae*. — Bull. Appl. Bot. Plant-breed. XIII; 2: 3—14.

26. LUND, S. og KIAERSKOU. 1884. En monografisk Skildring af Havekaalens, Rybsens og Rapsens Kulturformer. — Landbrugets Kulturplanter 4: 89—203.
27. — og — 1885. Morfologisk-anatomisk Beskrivelse af *Brassica oleracea* L., *B. campestris* (L.) og B. *napus* (L.) samt Redegjørelse for Bestøvnings- og Dyrkningsforsøg med disse Arter. — Bot. Tidsskrift 15: 1—150.
28. MAC KEY, J. 1954. The taxonomy of hexaploid wheat. — Svensk Bot. Tidskr. 48. (In the press.)
29. MANSFELD, R. 1953. Zur allgemeinen Systematik der Kulturpflanzen. I. — Die Kulturpflanzen I: 138—155.
30. MAYR, E. 1940. Speciation phenomena in birds. — Amer. Nat. 74: 249—278.
31. — 1942. Systematics and the origin of species. — New York.
32. MOHAMMAD, A. 1935. Pollination studies in toria (*Brassica napus* L. var. *dichotoma*, PRAIN) and sarson (*Brassica campestris* L. var. *sarson*, PRAIN). — Indian Journ. Agr. Sci. 5: 125—154.
33. MOHAMMAD, A. and SIKKA, S. M. 1937. Breeding investigations in some of the oleiferous *Brassicae* of the Punjab. — Indian Journ. Agr. Sci. 7: 849—861.
34. — and — 1940. Pseudogamy in the genus *Brassica*. — Curr. Sci. 9: 280—282.
35. MOHAMMAD, A., SINGH, R. D. and ALAM, Z. 1931. Some breeding investigations on toria (*Brassica napus* L. var. *dichotoma* PRAIN) and sarson (*Brassica campestris* L. var. *sarson* PRAIN). — Indian Journ. Agr. Sci. 1: 109—136.
36. MORINAGA, T. 1928. Preliminary note on interspecific hybridization in *Brassica*. — Proc. of Imp. Acad. 4: 620—622.
37. — 1929 a. Interspecific hybridization in *Brassica*. I. The cytology of F_1 hybrids of *B. Napella* and various other species with 10 chromosomes. — Cytologia 1: 16—27.
38. — 1929 b. Interspecific hybridization in *Brassica*. II. The cytology of F_1 hybrids of *B. cernua* and various other species with 10 chromosomes. — Jap. Journ. Bot. 4: 277—289.
39. — 1934. Interspecific hybridization in *Brassica*. VI. The cytology of F_1 hybrids of *B. juncea* and *B. nigra*. — Cytologia 6: 62—67.
40. MÜNTZING, A. 1929. Cases of partial sterility in crosses within a Linnean species. — Hereditas XII: 297—319.
41. — 1938. Sterility and chromosome pairing in intraspecific *Galeopsis* hybrids. — Hereditas XXIV: 117—188.
42. MÜNTZING, A., TEDIN, O. and TURESSON, G. 1931. Field studies and experimental methods in taxonomy. — Hereditas XV: 1—12.
43. MUSIL, A. F. 1948. Distinguishing the species of *Brassica* by their seed. — U. S. Dept. Agr. Misc. Publ. No. 634: 1—35.
44. NAGAI, K. and SASAOKA, T. 1930. The number of chromosomes in cultivated *Brassica*. — Jap. Journ. Genet. V: 151—158.
45. NILSSON, HERIBERT. 1930. Synthetische Bastardierungsversuche in der Gattung *Salix*. — Lunds Univ. Årsskr., N. F., Avd. 2, Bd 27, Nr 4, 96 pp.
46. OLSSON, G. 1953. Undersökning av självfertiliteten hos artificiell raps. — Lantbruksakad. Tidskr. 92: 394—402.
47. PEARSON, O. H. 1928. A suggested classification of the genus *Brassica*. — Proc. Amer. Soc. Hort. Sci. 25: 105—110.

48. PRAIN, D. 1898. The mustards cultivated in Bengal. — Agr. Ledger 5: 1—80.
49. RICHHARIA, R. H. 1937. Cytological investigation of 10-chromosome species of *Brassica* and their F_1 hybrids. — Journ. Genet. 34: 45—55.
50. SASAOKA, T. 1930. Karyological observations in different interspecific hybrids of *Brassica*. — Jap. Journ. Genet. VI: 20—32.
51. SCHULZ, O. E. 1919. *Cruciferae-Brassiceae*. — In ENGLER: Das Pflanzenreich IV, 105, Heft 70: 1—69.
52. — 1936. *Cruciferae* : *Brassica*. — In ASCHERSON und GRAEBNER: Synopsis der Mitteleuropäischen Flora V, 4: 6—56.
53. SHIMOTOMAI, N. 1925. A karyological study of *Brassica*. I. — Bot. Mag. Tokyo. 39: 122—127.
54. SIKKA, S. M. 1940. Cytogenetics of *Brassica* hybrids and species. — Journ. Genet. 40: 441—569.
55. SIMS, H. J. 1948. Wild Turnip — a serious Mallee weed. — Victoria Dept. Agr. Journ. 46, 2: 82—86.
56. SINSKAIA, E. 1927. Geno-systematical investigations of cultivated *Brassica*. — Bull. Appl. Bot. Plant-breed. XVII, 1: 3—166.
57. — 1928. The oleiferous plants and root crops of the family *Cruciferae*. — Bull. Appl. Bot. Plant-breed. XIX, 3: 1—648.
58. STEBBINS, G. L. 1950. Variation and evolution in plants. — New York.
59. STOUT, A. B. 1931. Pollen-tube behavior in *Brassica pekinensis* with reference to self-incompatibility in fertilization. — Amer. Journ. Bot. 18: 686—695.
60. SUN, V. G. 1946. The evolution of taxonomic characters of cultivated *Brassica* with a key to species and varieties. I and II. — Bull. Torrey Bot. Club 73: 244—281 and 370—377.
61. TERASAWA, Y. and SHIMOTOMAI, N. 1928. Bastardierungsversuche bei *Brassica* und *Raphanus*. — Sci. Rep. Tohoku Imp. Univ., Ser. IV (Biology) 3: 827—841.
62. U, N. 1935. Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. — Jap. Journ. Bot. 7: 389—452.

CONTENTS.

Material and methods 398
Results 401
Crosses including *B. Tournefortii* GOUAN 401
Crosses between *B. campestris*, *chinensis*, *pekinensis*, *narinosa*, and *nipposinica* 401
Discussion 410
Summary 415
Literature cited 416

SPECTROPHOTOMETRIC ANALYSIS OF ACETO-CARMINE SOLUTIONS

BY *A. LIMA-DE-FARIA* AND *S. BOSE*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

CARMINE is a natural dye obtained from the tropical insect *Coccus cacti*. By grinding and extracting the dried bodies of the females of this insect a dye is obtained known as cochineal. On treatment with alum this solution yields a product known as carmine (CONN, 1946). The commercial product is essentially a combination of carminic acid with aluminum, calcium and albuminoids (LANGERON, 1934). The exact chemical structure of carminic acid is somewhat uncertain.

As a consequence, commercial samples of carmine vary widely, and when a new sample is obtained two main questions arise: (1) what will be the properties of the solution obtained from the new sample, and (2) how to obtain the best solution from that sample? It is common practice to try to answer the first question by a *visual* inspection of the solution obtained or simply by applying it to chromosomes and observing the result. Such a process may give some information but this is incomplete. In order to get a solution of good quality, cytologists, as a rule, content themselves with making a »saturated» solution of the dye. Such a »saturated» solution, which is made, in many cases, by diluting 0,5 gm. of carmine in 100 c.c. of 45 % acetic acid (DARLINGTON and LA COUR, 1947; SMITH, 1947) is of limited use.

In an attempt to overcome these difficulties it was considered necessary to make not only a study of the dye from different firms and countries but also to have a reliable method for the comparison of the solutions obtained.

The carmine used in this study came from two English and two American firms: G. T. Gurr, London; Hopkin and Williams Ltd., Essex, England; National Aniline Division, New York (commission certified stain); S. B. Penick and Co., U. S. A. No German dye sources were used in this study owing to difficulties in obtaining samples of carmine from Germany.

Five solutions were made from each sample. The solutions were prepared by boiling 200 c.c. of 45 % acetic acid containing respectively 1, 2, 4, 8 and 16 gm. of carmine, the result being a series of solutions

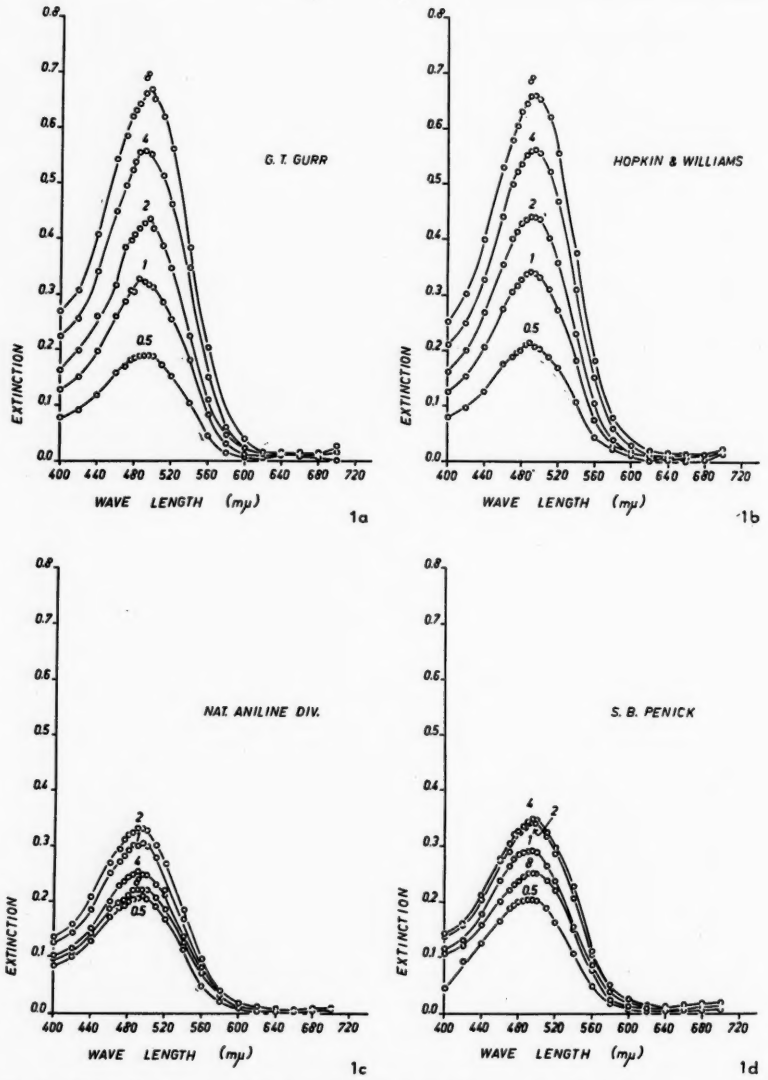


Fig. 1. Absorption curves of aceto-carmine solutions made from four different dye sources. A series of five initial concentrations (0,5—8 %) were used in each case.

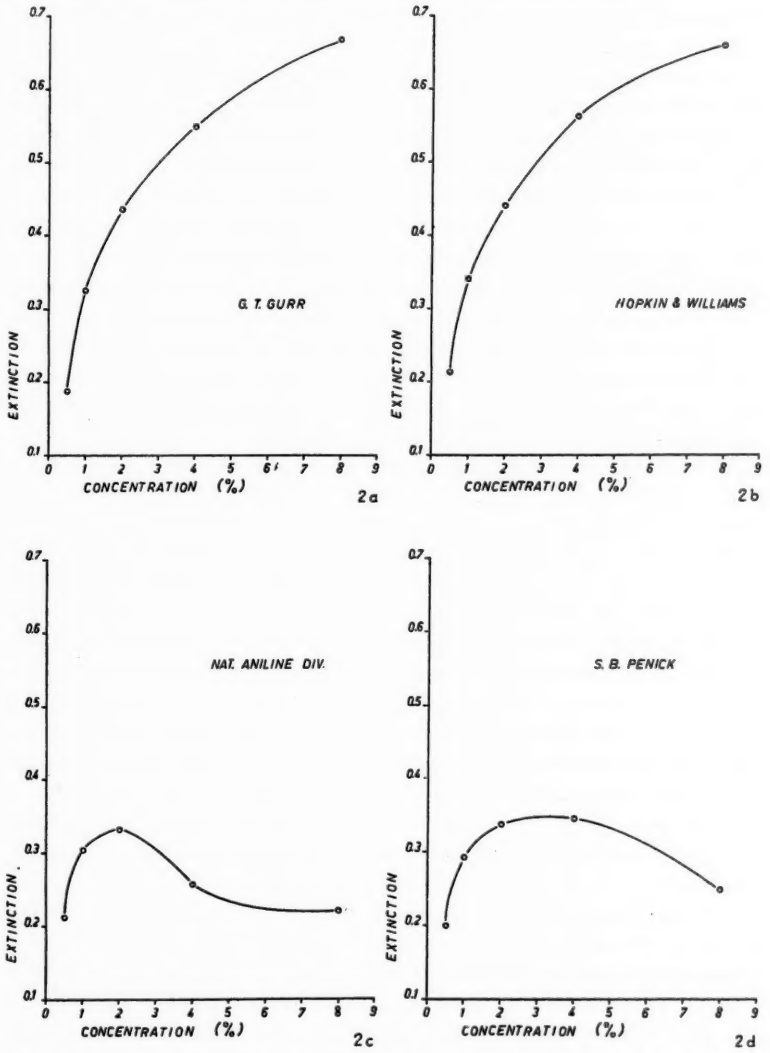


Fig. 2. Relationship between maximum absorption and concentration in four different carmine dye sources. Data extracted from Fig. 1.

with concentrations of 0,5, 1, 2, 4 and 8 %. To avoid any change in concentration during boiling, a boiling flask with a reflux condenser was used, and the boiling was prolonged for two hours to permit a full elution of the powder. The aceto-carmine solutions were then set aside for two days, and after that time decanted and filtered. All the solutions were prepared under rigorously similar conditions.

All sixteen solutions with concentrations of 1, 2, 4 and 8 % prepared from the four dye sources left an obvious deposit after filtration. The four 0,5 % solutions left in some cases only a slight deposit.

No iron was added to the aceto-carmine solutions in order to avoid introducing any extraneous source of error.

All the solutions were too opaque to be used directly for spectrophotometric analysis. The dilution of 1 c.c. of a standard solution into 200 c.c. of 45 % acetic acid was found to provide a solution of the desired transparency. All the twenty standard solutions were analysed in this way. A photoelectric spectrophotometer was used to avoid personal bias in the readings. The instrument employed was a Unicam diffraction grating spectrophotometer from Unicam Instruments (Cambridge) Ltd. In the spectrophotometric analysis the solvent — 45 % acetic acid — was used as blank. The readings were made in the region of the visible between 400 and 700 millimicrons.

An inspection of the graphs (Fig. 1) reveals the following results:

(1) All the absorption curves have their maximum absorption in the same region of the spectrum, irrespective of concentration and origin of the dye. The maximum absorption zone is found to be between 480—500 millimicrons, which is situated within the absorption region of blue-green, complementary colour to orange-red.

(2) There is for each absorption curve a single zone on the spectrum at which light is absorbed to a greater extent than on either side of it.

(3) In the two English dye samples (G. T. Gurr and Hopkin and Williams) the intensity of the stain increased with the dye concentration whereas in the two American dye samples analysed (Nat. Aniline Division and S. B. Penick) the intensity was maximum in the 2 % and 4 % solutions, respectively, and decreased at higher concentrations (Figs. 1 and 2).

The fact that for all the different concentrations the region of maximum absorption lies in the same zone (480—500 millimicrons), is interpreted as indicating that the same substance or the same combination of substances is dissolved in all cases. Moreover, the carmine

samples studied are not heterogeneous mixtures as there is a single top for each curve.

The decrease of the stain intensity with increasing concentration, as observed in the case of the two American dye samples, is difficult to explain. In these dye samples the molecular combinations of carmine may form large aggregates which — when in large concentration — are difficult to break down and for this reason stay on the filter paper and do not pass into the solution.

The very striking similarity of the curves of the two English dye samples may be due to a similar method of preparation or a common source of the original dye stuff.

These results enable us to use carmine samples for cytological purposes on a more exact basis. Once a new sample is available, instead of a single »saturated» solution, a series of five or more solutions with different concentrations may be made and easily analysed in the photoelectric spectrophotometer. Such a procedure will rapidly give information about the properties of the new sample and will furnish a series of solutions with different stain intensities. The dilution by half of the 8 % solution from Hopkin and Williams furnishes a solution of weaker stain intensity than the 4 % standard solution obtained after boiling. Successive dilutions of the 8 % standard solution furnish weaker solutions than the original series obtained in the way described above. For this reason one should avoid making a single standard solution and then diluting it.

As different species and different stages require different stain intensities the use in cytological work of such a series is most convenient. Stages where the chromosomes are thin and long, as at pachytene, require a deep stain. At stages where the chromosomes have become thicker, such as diakinesis and metaphase I, weaker stains should be used. There is also a difference among plants with small, medium size, and long chromosomes. For instance, long and thick chromosomes like those of *Trillium erectum* require at metaphase I of meiosis a weaker stain than the chromosomes of rye at the same meiotic stage.

The subsequent introduction of iron into these aceto-carmine solutions results in a still larger number of hues. In this way the cytologist will have at his disposal a scale of iron-aceto-carmine solutions which will permit him to obtain easily the desired stain for a given species and a given stage.

SUMMARY.

Aceto-carmine solutions with concentrations ranging from 0,5 to 8 % were prepared under standardized conditions from two English and two American dye sources. These solutions were analysed, after convenient dilution, by means of a photoelectric spectrophotometer. All these solutions, although they contain in most cases an excess of dye, were found to have different stain intensities which increased with the initial concentration in the case of the two English dye samples. For all concentrations the maximum absorption was situated at 480—500 millimicrons. Such a series of concentrations furnishes information concerning the properties of the sample and is most useful in cytological work since different species and different stages require different stain intensities.

LITERATURE CITED.

1. CONN, H. J. 1946. Biological stains. — Geneva, N. Y., U. S. A. 346 p.
2. DARLINGTON, C. D. and LA COUR, L. F. 1947. The handling of chromosomes. — London. 180 p.
3. LANGERON, M. 1934. Precis de microscopie. — Paris. 1205 p.
4. SMITH, L. 1947. The acetocarmine smear technic. — Stain Technology 22: 17—31.

ON ACCESSORY CHROMOSOMES IN FESTUCA PRATENSIS

II. INHERITANCE OF THE STANDARD TYPE OF ACCESSORY CHROMOSOMES

BY *NILS OLOF BOSEMARK*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

THE present report deals with the inheritance of the *standard type* of accessory chromosomes in *Festuca pratensis* HUDS. (BOSEMARK, 1954) in different cross combinations. This type has been studied in crosses made by isolating together panicles in pollen-proof bags and by open pollination. In view of the rather pronounced self-sterility in *Festuca pratensis* (NILSSON, 1933) this method was chosen in favour of the more laborious controlled crosses. NILSSON found up to 11,3 per cent self-fertility in extreme cases; I have therefore always made control isolations of the plants involved in the crosses at the same time. From the number of seeds obtained in these isolations it has been possible to determine the degree of self-fertility, and plants in which self-fertility exceeded a low arbitrary limit (10 seeds per two isolated panicles) were excluded. In Table 1 some of the resultant data are given.

Most of the plants are highly self-sterile giving less than 5 seeds per two panicles. As the total number of flowers per panicle is about 150—200, the per cent seed set is very low. The results are in good agreement with those obtained by NILSSON.

As the first crosses already showed that non-disjunction of the accessory chromosomes takes place only in the pollen grain (BOSEMARK, 1950, 1954), my interest was centered on studying the effectiveness of this mechanism in plants of different origin. In such crosses of the type

TABLE 1. *Self-fertility in Festuca pratensis.*

Year	Number of plants with respective number of seeds from two isolated panicles												
	0	5	10	15	20	25	30	35	40	45	50	55	60
1949	40	13	4	7	1	3	1	1					
1950	62	15	4	6	2					1			1
Total	102	28	8	13	3	3	1	1	1			1	

TABLE 2. *Crosses of the type (14+1 acc.)×14 and 14×(14+1 acc.).*

Crosses	Parents Plant Nos. ♀	♂	Number of acc. chr. ♀	♂	F_1 Number of acc. chr. 0	1	2	3	Number of plants	M. acc. chr. per plant
K1/49	84×	86	1×	0	47	18			65	0,28
K2/49	86×	84	0×	1	50	20	30		100	0,80
K86/50	493×	492	1×	0	50	32	2	1	85	0,46
K85/50	492×	493	0×	1	52	3	35		90	0,81
K70/50	437×	436	1×	0	18	12		1	31	0,48
K69/50	436×	437	0×	1	36	16	8		60	0,53
K83/50	491×	492	1×	0	88	64	1	1	154	0,45
K84/50	492×	491	0×	1	21	17	7		45	0,69
K71/50	437×	438	1×	0	21	23			44	0,52
K72/50	438×	437	0×	1	21	16	1		38	0,47
K51/51	600×	599	1×	0	40	31			71	0,44
K50/51	599×	600	0×	1	39	4	21	3	67	0,82

14×(14+1 acc.) and 14×(14+2 acc.) self-fertility of the maternal parent does not affect the ratio between progeny plants having one and two accessory chromosomes.

Crosses of the type (14+1 acc.)×14 and 14×(14+1 acc.). — During the years 1949—1951 altogether 21 crosses (14+1 acc.)×14 and 22 in the reciprocal direction were analysed. Table 2 gives some examples from these crosses.

As seen from the data, there is no numerical increase of accessory chromosomes on the female side. On the male side, on the other hand, there is in some crosses a very marked increase in the number of plants with two accessory chromosomes, resulting in a mean number of accessories much higher than the 0,5 value, which would be expected without elimination and a mechanism for increase. The mean number of accessory chromosomes in crosses of the type 14×(14+1 acc.) may be affected by self-fertilization in the maternal parent, degree of meiotic elimination and frequency of non-disjunction in the paternal parent and by directed fertilization. The amount of self-fertilization was low in the plants used, and no evidence for directed fertilization found, although such a mechanism cannot be entirely excluded, especially as it has been shown to be at work in maize with accessory chromosomes (ROMAN, 1948). Be this as it may, the effect of all these processes together can be checked by comparing number of plants with and without accessory chromosomes. As seen from the table, there is a certain amount of

variation with respect to these figures in different crosses, but only in a few of them have the accessories been lost to a higher degree. A much more interesting variation is the one between number of plants with one and two accessories in the crosses 14×(14+1 acc.), where the figures tell us to what extent non-disjunction has taken place. The extremes are represented by K85/50 with 35 successful non-disjunctions out of 38 and K72/50 with only one successful out of 17. As the numbers to be compared are sometimes rather small, a test of heterogeneity for all available data from crosses of the type 14×(14+1 acc.) and 14×(14+2 acc.) has been made to ascertain the difference between plants in this respect (Table 3). In the table the figures for plants lacking accessories have been excluded.

The analysis shows a pronounced heterogeneity in all the years, and thus one can safely conclude that the non-disjunction mechanism is less effective in some plants than in others. The next question that arises is whether this depends on variation in external environment, inherent differences between different accessory chromosomes, or on differences in the internal environment provided for the

TABLE 3. *Number of plants with one and two accessory chromosomes in crosses of the types 14×(14+1 acc.) and 14×(14+2 acc.) from different years.*

1949			1950			1951		
Number of plants with		Per cent plants with	Number of plants with		Per cent plants with	Number of plants with		Per cent plants with
1 acc.	2 acc.	2 acc.	1 acc.	2 acc.	2 acc.	1 acc.	2 acc.	2 acc.
20	30	60,0	11	4	26,7	5	20	80,0
2	19	90,5	16	8	33,3	0	31	100 0
3	6	66,7	3	35	92,1	17	62	78,5
4	13	76,5	4	10	71,4	5	31	86,1
9	7	43,8	5	13	72,2	21	48	69,6
0	6	100,0	7	64	90,1	4	21	84,0
0	5	100,0	10	58	85,3			
1	7	87,5	35	53	60,2			
10	12	54,5	16	1	5,9			
3	9	75,0	28	9	24,3			
0	32	100,0	17	7	29,2			
8	8	50 0						
4	13	76,5						
1	40	97,6						
65	207	76,1	152	262	63,3	52	213	80,4
$\chi^2=55,246$			$\chi^2=129,400$			$\chi^2=13,822$		
$P < 0,001$			$P < 0,001$			$0,05 > P > 0,01$		

TABLE 4. *Number of plants with one and two accessory chromosomes in crosses of the type 14×(14+1 acc.) and 14×(14+2 acc.) made on the same day.*

Three crosses in the field (14/6 1949)			Five crosses in the greenhouse (17/6 1951)		
Number of plants with		Per cent plants with	Number of plants with		Per cent plants with
1 acc.	2 acc.	2 acc.	1 acc.	2 acc.	2 acc.
20	30	60,0	5	20	80,0
2	19	90,5	0	31	100,0
0	32	100,0	17	62	78,5
			5	31	86,1
			21	48	69,6
22	81	78,6	48	192	80,0
	$\chi^2=20{,}785$			$\chi^2=13{,}402$	
	$P<0{,}001$			$P=0{,}01$	

accessories by their host plants. This question I have tried to answer by repeating crosses in the same year, as well as in different years, and by crossing progeny plants from parents with well and poorly functioning non-disjunction mechanism with new test plants without accessory chromosomes. By repeating the same cross one should be able to find out the influence of the external environment; crossing progeny plants may reveal inherent differences between accessory chromosomes. I have not been able to obtain statistically significant data from these crosses. Although the number of plants has been relatively small the third alternative is indicated; the effectiveness of the non-disjunction mechanism is conditioned primarily by the internal plant environment. This hypothesis has been tested by comparing crosses made on the same day (Table 4).

The 1949 crosses were made in the experimental field, those in 1951 in a greenhouse to which all the plants were brought on the same day, well before meiosis had started. A χ^2-analysis of the data shows a marked heterogeneity in both years. This strongly suggests that other factors than variation in external conditions bring about the variation observed. This is in conformity with the cytological findings (BOSEMARK, 1954), where it was shown that univalent accessory chromosomes in two plants having to some degree derived their accessories from the same mother plant reacted quite differently at anaphase I in meiosis, though they were growing nearly side by side in the field. It is thus highly probable that the physiology of the host plant is of great importance also with respect to the non-disjunction. This does not, of

course, exclude the possibility that the external environment has a modifying influence, as is indeed very probable, or that accessory chromosomes of different origin are really different with respect to their capacity for non-disjunction. A better idea of the general effectiveness of the non-disjunction mechanism in plants with one accessory chromosome can be derived from Table 5, where all the data from crosses of the types 14×(14+1 acc.) and (14+1 acc.)×14 are condensed. It should be pointed out that nearly all the plants used in these crosses were collected in natural populations covering most of the distribution range of the species in Sweden. The material thus is fairly representative and the results can be looked upon as a good mean for Swedish populations.

As seen, the mean values for the total material within the different years agree quite well with each other except for the crosses in the direction 14×(14+1 acc.) in 1950. The mean accessory chromosome number is here 0,58 corresponding to 0,72 in 1949 and 0,67 in 1951. The difference is still more obvious if we compare the figures for one and two accessory chromosomes, respectively. Then we get 52 to 146 in 1949 and 100 to 87 in 1950. The heterogeneity between all three years gives a χ^2-value of 30,013 with a p-value smaller than 0,001. This difference could depend on differences between the materials used in the different years or between the environmental conditions. Although some of the plants used in the crosses in 1950 originated from more northern localities than did the material in 1949, crosses involving plants from southern Sweden gave more or less the same results this year. The weather conditions during the period of flowering in 1950 were very bad, with heavy rainfalls, hail and storm; I am inclined to consider this partly responsible for the bad functioning of the non-disjunction, al-

TABLE 5. *Summary of all crosses 14×(14+1 acc.) and (14+1 acc.)×14.*

Year	Parents Number of acc. chr. ♀ ♂	Number of crosses	F_1 Number of acc. chr. 0	1	2	3	Number of plants	M. acc. chr. per plant
1949	1×0	12	304	197	7	2	510	0,43
»	0×1	11	279	52	146		477	0,72
1950	1×0	7	307	200	5	3	515	0,43
»	0×1	8	282	100	87		469	0,58
1951	1×0	2	73	48			121	0,40
»	0×1	3	107	25	42	3	177	0,67
Total	1×0	21	684	445	12	5	1146	0,42
	0×1	22	668	177	275	3	1123	0,66

TABLE 6. *Crosses of the type (14+2 acc.)×14 and 14×(14+2 acc.).*

Crosses	Parents Plant Nos. ♀ ♂	Number of acc. chr. ♀ ♂	F_1 Number of acc. chr. 0	1	2	3	4	Number of plants	M. acc. chr. per plant
K108/49	171×170	2×0	2	31	1	2		36	1,08
K109/49	170×171	0×2	9	1	40			50	1,62
K2/50	298×297	2×0	8	77	2	1		88	0,95
K1/50	297×298	0×2	7	7	64		12	90	2,03
K66/50	384×383	2×0	4	76		1		81	0,98
K65/50	383×384	0×2	14	35	53		2	104	1,43
K7/51	2/49 : 4×290	2×0	3	36				39	0,92
K8/51	290×2/49 : 4	0×2	5	17	62		4	88	1,78
K5/51	20/49 : 4×22/49 : 4	2×0	1	75	4			80	1,04
K6/51	22/49 : 4×20/49 : 4	0×2	3	21	48			72	1,63

though this could not be proved statistically in the repeated crosses. Thus the effectiveness of non-disjunction is probably the result of an interaction of external as well as internal factors. The loss of accessory chromosomes in both directions amounts to about twenty per cent which must be considered a low figure as in general 70 per cent of the accessories divide at anaphase I in meiosis (BOSEMARK, 1954). The data now presented also show that it is very dangerous to draw any conclusions with respect to the general effectiveness of the non-disjunction mechanism from a material limited with respect to number of crosses and origin.

TABLE 7. *Summary of all crosses (14+2 acc.)×14 and 14×(14+2 acc.)*

Year	Parents Number of acc. chr. ♀ ♂	Number of crosses	F_1 Number of acc. chr. 0	1	2	3	4	5	Number of plants	M. acc. chr. per plant
1949	2×0	6	15	175	4	13	2	1	210	1,12
»	0×2	3	11	13	61				85	1,59
1950	2×0	3	16	211	4	7			238	1,01
»	0×2	3	31	52	175	15			273	1,64
1951	2×0	4	8	215	12				235	1,02
»	0×2	4	20	43	171	1	4		239	1,69
Total	2×0	13	39	601	20	20	2	1	683	1,05
	0×2	10	62	108	407	16	4		597	1,65

Crosses of the type (14+2 acc.)×14 and 14×(14+2 acc.). — As already mentioned, a number of crosses of this type have been made, and some results are given below.

Here the greater regularity in the transmission of the accessory chromosomes at meiosis in plants with two accessories is reflected; the number of plants lacking accessories is low in both directions. With respect to non-disjunction the same variation as for the crosses involving plants with one accessory chromosome is present. In Table 7 the results from all the analysed crosses of this type are summarized.

Also here the difference between years with respect to the non-disjunction is present though less marked. The ratios between numbers of plants with one and two accessory chromosomes for the respective years are: 1949, 13/61=0,21; 1950, 67/190=0,35, and 1951, 44/174=0,25.

Crosses between plants with higher numbers of accessory chromosomes. — In Table 8 some of the data obtained in crossing plants with higher numbers of accessory chromosomes are given.

It is striking that here some of the reciprocal crosses give about the same mean number of accessory chromosomes. In some crosses, for instance K117/49 and K73/49, this may depend partly on self-fertilization, although only plant 136 gave some seeds under isolation, whereas in K14/50 self-fertilization of the mother plant cannot be responsible for the low average number of accessory chromosomes. Here meiotic elimination and a less effective non-disjunction mechanism are probably responsible for the result obtained. From the crosses K17/51 and K18/51 it is, however, clear that high numbers of accessory chromosomes do not necessarily mean high elimination and a poorly functioning non-disjunction mechanism. With respect to meiotic elimination this has also been cytologically verified (BOSEMARK, 1954). Also with plants carrying still higher numbers of accessory chromosomes several crosses have been made, isolating together panicles in the way previously described. Most of these crosses gave very few seeds, and the results with respect to chromosome numbers are of little or no significance. For this reason I planted out in 1950 on an isolated spot eleven plants having from 5 to 8 accessory chromosomes and let them flower openly together. There was no marked difference in earliness between the plants, and the different progenies can thus be considered more or less the result of a polycross. From each of the plants all the panicles were thrashed, the seeds obtained were mixed well and a sample of 100 seeds taken out from each of the mother plants. The result of the determinations of the chromosome numbers in these samples is seen in Table 9. The discrepancy

TABLE 8. *Crosses involving plants with higher numbers of accessory chromosomes.*

Crosses	Parents: Plant numbers ♀	♂	Number of acc. chr. ♀	♂	F_1: Number of acc. chr. 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Number of plants	M. acc. chr. per plant
K13/50	820	821	1	0	1	16	13	4	2	1										37	1,81
K14/50	821	820	0	4		12	2	2	4											20	1,90
K43/49	124	145	0	4		8	19	5	21	1	1	1	1							57	3,00
K118/49	214	213	4	1		2	13	9	14	1	1	1								41	3,15
K117/49	213	214	1	4	1	4	8	2	4	16										35	3,49
K74/49	208	136	4	3				5	9	5	12	2	1							34	5,00
K73/49	136	208	3	4			2	8	10	15	8		1							44	4,52
K11/51	289	K58/49 : 2	0	5	4	3	4		24	8	12	1	1		1					58	4,21
K17/51	212	K34/48 : 1	7	0		1	16	62[1]	43	11	2	1								136[3]	3,42
K18/51	K34/48 : 1	212	0	7					6	2	5	2	1							16	5,38
K34/51	1151	K56/49 : 5	5	7				3	7[2]	7	17[1]	25[1]	11[1]	11	4	1	2	5	1	94[4]	7,34

[1] = One plant in addition one very small acc. chr.
[2] = » » had ring chromosomes.
[3] = In addition one spontaneous triploid.
[4] = » » » » tetraploid.

TABLE 9. *Chromosome numbers in progenies from eleven plants intercrossed in 1950.*

Mother plants		F_1 Number of accessory chromosomes																
Plant Nos.	Number of acc. chr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Number of plants	M. acc. chr. per plant
14/48 : 1	8					2	2	11	18	5	12	4	3	6	4	1	1	69	9,46
11/48 : 8	8			1	3	8	9[1]	10	11	4	4	7	1	1	3	1	1	64	8,09
1108	7			2	5	6	6	6	11	7	9[7]	2	1	1	1			57[5]	7,65
1102	6		1	2[1]	6	3	5	5	13	11	3		1	2	1			53	7,49
1016	6		2	6	1	8	14[2]	8	14	9	2	2	5	2				73	7,22
1014	6	1	1	4	3	7	11	6	9	4	1		3		1			51	6,63
5/18 : 10	6			1	4	13[1]	6	12	11[6]	7	2	1						57	6,77
12/48 : 6	5	1	1	7	6	11	15[1,2]	14	7	6	2							70	6,01
14/48 : 8	5	1	5	6	10	13	10	13	5	1	2		3	1				70	5,76
1151	5	1	1	2	1	7	17[1]	16	7	5[7]	9	1	1	2			1	71[4]	7,30
997	5	3	21[3]	21	33[3]	17	26	12	13	7	2	4						159	5,01

[1] = One plant in addition one small acc. chr.
[2] = » » » » two » » »
[3] = » » » » one very small acc. chr.
[4] = In addition one spontaneous triploid.
[5] = » » » » tetraploid.
[6] = One plant had ring chromosomes.
[7] = » » » a true fragment.

between the number of seeds in the original seed sample and the numbers in the table is the result of poor germination and the fact that chromosome counts could not be made in some of the plants. That these seeds, not germinating in the germination apparatus in 1951, did not represent any special chromosome numbers has been shown in 1952 where a new sample of 100 seeds from the mother plant 997 germinating in a bench in the greenhouse gave 98 plants with practically the same chromosome number distribution as in 1951. These plants have therefore been included in Table 9.

The increase in average numbers of accessory chromosomes compared to the mother plants is here less marked than in the crosses between plants with lower numbers of accessories, but in no case is the average lower. One of the purposes of this intercross was to find out how high a number of accessory chromosomes could be obtained, and for the same reason I similarly intercrossed six plants with 13, 14 and 15 accessories in an isolation cell in the greenhouse in 1952. Here I have determined the chromosome numbers in 59 progeny plants from one mother plant having 15 accessory chromosomes (Table 10).

The range of accessory chromosome numbers here is from 4—19 with a mean value of 11,83. Without meiotic elimination and with a well functioning non-disjunction mechanism one should expect much higher numbers of accessory chromosomes, but as this is not the case and as all the plants had very poor seed-set, I think that these numbers represent the maximum that can be produced. In this connection I might point to the fact that in more than 600 plants with accessory chromosomes collected in natural populations in Sweden and England not one had more than seven accessories and only about ten per cent had more than two.

TABLE 10. *Chromosome numbers in the progeny from a plant with 2n=14+15 acc. intercrossed with five plants having 13, 14 and 15 accessory chromosomes.*

	Number of accessory chromosomes																Total	M. acc. chr. per plant
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		
Number of plants	1	—	4[2]	3	3	3	5	7	5	8	6	3	6	3[1]	—	1	58[3,4]	11,83

[1] = One plant in addition one small acc. chr.
[2] = » » » » two very small acc. chr.
[3] = In addition one spontaneous triploid.
[4] = » » » » tetraploid.

In 1952 also a number of pair crosses involving plants with very high numbers of accessory chromosomes were made, but in all cases the plants were so highly sterile that only a few seeds were obtained from 15—20 panicles.

Another thing that can be studied in Table 10 and the two previous ones is that with increasing numbers of accessory chromosomes in the mothers the number of plants in the progenies with smaller types of accessories, true fragment chromosomes and ring chromosomes as well as triploids and tetraploids increases. This is in conformity with the cytological observations (BOSEMARK, 1954) where it was shown that disturbances in meiosis and pollen mitosis are frequent in these plants. With respect to the smaller types of accessory chromosomes these should, of course, be more common in these plants also for mere numerical reasons.

Discussion and conclusion. — The present investigation has clearly shown that the non-disjunction of the accessory chromosomes in *Festuca pratensis* (BOSEMARK, 1950, 1954) takes place only in the pollen grain. The situation is the same as in *Anthoxanthum* (ÖSTERGREN, 1947), whereas in rye the mechanism is at work also in the embryo-sac (MÜNTZING, 1945; HÅKANSSON, 1948). In maize there is non-disjunction only on the male side, but here it occurs at the second division in the pollen grain and is accompanied by directed fertilization (ROMAN, 1947, 1948, 1950), whereas in *Sorghum*, finally, the non-disjunction takes place in supernumerary divisions of the vegetative nucleus (DARLINGTON and THOMAS, 1941).

Even with respect to the relatively low meiotic elimination of the accessory chromosomes the similarity between *Festuca* and *Anthoxanthum* is striking. In rye the meiotic elimination is often pronounced, especially in »Östgöta Gråråg» (MÜNTZING, 1945). The data for the transmission of the accessory chromosomes in maize presented by RANDOLPH (1941) do not indicate a very high degree of loss in crosses involving low numbers, which, as pointed out by ÖSTERGREN (1947), are the most important for maintenance in a population. MÜNTZING (1946) considers the mechanism for numerical increase of the accessories in rye sufficient for their maintenance in the populations, and does not find it necessary to postulate a positive selective value for the accessory chromosomes. The same opinion is held by ÖSTERGREN (1947), whereas DARLINGTON and UPCOTT (1941) from their data drew the conclusion that the accessory chromosomes in maize must possess some kind of positive effect. In *Festuca pratensis* the meiotic elimination of the ac-

cessory chromosomes in plants with low numbers is relatively low, and the general effectiveness of the non-disjunction mechanism must be considered quite good. Irrespective of the opinion held with regard to a positive selective value of low numbers of accessory chromosomes in this material, there is thus no evidence that the accessories are not capable of maintaining or even spreading themselves in natural populations.

SUMMARY.

(1) The inheritance of the standard type of accessory chromosomes in *Festuca pratensis* HUDS. was studied in different cross combinations.

(2) Non-disjunction of the accessory chromosomes occurs only in the pollen grain.

(3) The effectiveness of this mechanism for numerical increase of the accessories varies between individuals, probably largely depending on the genotype of the host plant.

(4) The meiotic elimination as reflected by the crosses is fairly moderate even in crosses involving plants with only one accessory chromosome.

(5) The progenies after crosses involving plants with high numbers of accessory chromosomes reflect the cytological disturbances in these plants.

(6) The highest number of accessory chromosomes obtained in a cross is nineteen and due to the increasing sterility and cytological disturbances with increasing numbers of accessories this number is considered to represent about the maximum that can be obtained.

(7) The general effectiveness of the non-disjunction mechanism in combination with the fairly low meiotic elimination gives the accessory chromosomes quite good possibilities of maintaining themselves and even spreading in a population even if a positive selective value of low numbers of accessories should not be at hand.

LITERATURE CITED.

1. BOSEMARK, N. O. 1950. Accessory chromosomes in *Festuca pratensis* HUDS. — Hereditas XXXVI: 366—368.
2. — 1954. On accessory chromosomes in *Festuca pratensis*. I. Cytological investigations. — Hereditas XL: 346—376.
3. DARLINGTON, C. D. and THOMAS, P. T. 1941. Morbid mitosis and the activity of inert chromosomes in *Sorghum*. — Proc. Roy. Soc. London, B 130: 127—150.

4. DARLINGTON, C. D. and UPCOTT, M. B. 1941. The activity of inert chromosomes in *Zea mays*. — Journ. of Gen. 41: 275—296.
5. HÅKANSSON, A. 1948. Behaviour of accessory rye chromosomes in the embryo-sac. — Hereditas XXXIV: 35—59.
6. MÜNTZING, A. 1945. Cytological studies of extra fragment chromosomes in rye. II. Transmission and multiplication of standard-fragments and iso-fragments. — Hereditas XXXI: 457—477.
7. — 1946. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at the pollen mitosis. — Hereditas XXXII: 97—119.
8. NILSSON, F. 1933. Studies in fertility and inbreeding in some herbage grasses. — Hereditas XIX: 1—162.
9. RANDOLPH, L. F. 1941. Genetic characteristics of the B chromosomes in maize. — Genetics 26: 608—631.
10. ROMAN, H. 1947. Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. — Genetics 32: 391—409.
11. — 1948. Directed fertilization in maize. — Proc. Nat. Acad. Sci. 34: 36—42.
12. — 1950. Factors affecting mitotic nondisjunction in maize. Abstract 1949 Meetings of the Genetics Society of America. — Genetics 35: 132—133.
13. ÖSTERGREN, G. 1947. Heterochromatic B-chromosomes in *Anthoxanthum*. — Hereditas XXXIII: 261—296.

CONTENTS.

Crosses of the type (14+1 acc.)×14 and 14×(14+1 acc.) 426
Crosses of the type (14+2 acc.)×14 and 14×(14+2 acc.) 431
Crosses between plants with higher number of accessory chromosomes 431
Discussion and conclusion 435
Summary 436
Literature cited 436

INTRASPECIFIC STERILITY IN OATS

BY *ÅKE ÅKERMAN* AND *ARNE HAGBERG*
THE SWEDISH SEED ASSOCIATION, SVALÖF, SWEDEN

I. INTRODUCTION.

WHEN plant breeding was started at Svalöf in 1886, several different oat varieties were grown in Sweden (ATTERBERG, 1889). Some of them were whitehulled, others were blackhulled. Among the whitehulled ones, the varieties belonging to the type Northern white oats were dominant in the southern and western parts of Sweden. In Scania and adjacent provinces it was, however, already superseded by Probstei oats, an indigenous variety from Holstein in Northern Germany. Probstei oats outyielded the Northern white oats, had a more stiff straw and a better grain quality. Selection of individual progeny strains, »pedigrees», was made on a large scale in the Probstei oats (NILSSON-EHLE, 1906), and as especially outstanding pedigree strains Golden Rain (Guldregn) (HJALMAR NILSSON, 1903) and Victory (Seger) (NILSSON-EHLE, 1908) are well known.

In the eastern and northern parts of Sweden blackhulled varieties of oats were most commonly grown. In the southern part of this district, with its centre in the region around the lake Mälaren, they used a special type, »the black oats of Central Sweden», and beside this an imported black, plumed oats (»Scotch» or »Tartary»). In the greater part of Norrland (=the northern half-part of Sweden), however, there was grown from of old an early type af black oats adapted to the growth conditions prevailing there and closely related to the black oats grown in northern Finland.

The black oats of central Sweden (the varieties »Visingsö», the black »Risp» oat from Kolbäck and »Roslags» were the most commonly used) were grown far into this century on the stiff clays of central Sweden (MUNDT-PETERSEN, 1937). These indigenous varieties and the high-bred ones now mostly used, *viz.* Bell I—III and Great Mogul I and II, are characterized, among other things, by relatively late shooting and by good resistance to drought. Further, they have a better tillering capacity and are usually less damaged by the frit fly (*Oscinis frit*) than, for instance, Victory and Golden Rain (ÅKERMAN, 1951 b). With those characters the black oats of central Sweden are better adapted to the

dry and heavy clay soils in this region of Sweden than are the Probstei varieties (*cf.* NILSSON-EHLE, 1906 and ÅKERMAN, 1951 a). Sowing is often relatively late on these soils and, thus, followed by severe attacks of the larvae of the frit fly.

With the exception of Fyris, these black oats, however, have a relatively poor grain quality. Thus, the hectolitre weight is low and so is the content of grain (content of husks is high) (TORSSELL and MAC KEY, 1947). Since a long time it has been attempted to combine the good characters of the Probstei varieties with those of the black oats. Crosses were carried out very early. Thus, two years before the rediscovery of the Mendelian laws, the F_1 generation of the cross Golden Rain × Bell I was growing in the fields at Svalöf. From the year 1900 on NILSSON-EHLE worked with this and other crosses of the same type especially from a theoretical point of view.

II. THE FERTILITY IN TWO WHITE × BLACK OAT CROSSES.

In the year 1913 two crosses were made, Golden Rain × Bell II and Victory × Bell II. ÅKERMAN took over the material as F_2 in 1915 and found that many of the F_2 plants were partially sterile. The parental material had a seed setting of, for Golden Rain 96,4 %, for Victory 95,5 %, and for Bell II 91,8 %.

The F_2 plants selected from the first cross as promising from different points of view had a seed setting of 87,7 %, on an average. They were grouped into 4 classes according to grain colour but there were no differences in fertility between these classes. The non-selected plants had an average seed setting of 84,9 %. In the second cross, Victory × Bell II, the fertility conditions were about the same. The selected plants had 88,9 % seed setting and the other group 86,1 %. The difference between selected and non-selected plants is significant, $t = 5{,}86$ and $P < 0{,}001$. (Table 1.)

Thus, the progeny plants from these crosses showed a lower seed setting than the parents and it was supposed that this was caused by some kind of irregularity at meiosis or at fertilization and embryo development. Also in later generations partial sterility was observed, although no detailed data are available.

During the four decades ÅKERMAN has handled the oat breeding at Svalöf a very great number of crosses between white and black oats have been carried out in order, first, to get a black oat with good grain

quality, secondly, to get a white oat with the growth rhythm of the black oat. The progenies from all these crosses have shown partial sterility or decreased seed setting. Very often this partial sterility has caused rejection in the selection work. No satisfactory combinations of the characters desired have as yet been obtained from this type of crosses. As a matter of fact some black oat varieties have been bred, Sirius I and Extra Bell I, which have a good grain quality, but none of them have the growth rhythm of the black oat and the adaptability to the dry clay soils of central Sweden. Not even varieties derived from the back crosses of these two varieties to the black oats Engelbrekt and Great Mogul II, *viz.*, Sirius II and Extra Bell II, respectively, have the growth rhythm of the black oats.

The work carried out to combine the white hull with the growth rhythm of the black oat has not been successful either; this in spite of the fact that hull colour is monofactorially inherited. Some of the progenies, however, have a greyish or brown hull colour, different from the Probstei varieties. This indicates a more complicated inheritance of hull colour.

The experiencies related above indicate that from a genetical point of view the two types of oats are rather differentiated. This is in good agreement with the results obtained by FRÖIER (1946). He found in his mutation experiments that the two groups of oats have essential differences in the genes regulating the chlorophyll formation.

TABLE 1. *Seed setting in F_2 plants of crosses between black and white oat varieties 1915.*

		Per cent of seed setting																
		68	70	72	74	76	78	80	82	84	86	88	90	92	94	96	98	100 M
Golden Rain×Bell II																		
As good selected	a							1	3	2	2	1	2	4	1			88,3
plants, grouped	b						1		1	2	3	3	3	6	1			89,3
according to grain	c								3	4	4	2	2		1	1		87,6
colour	d	1					2		4	4	6	2	7		2			86,6
Non-selected plants	e		1	1		1	2	2	1	3	1	8	2	1				84,9
Bell II×Victory																		
As good selected	a									1	1	3	2		2			90,1
plants, grouped	b								1	2	5	4	2		1			88,1
according to grain	c				1		1		1	3	2	4	2	3	2			88,1
colour	d					1	1			4	3	7	8	6	3			89,4
Non-selected plants	e			1	1	2	5	11	14	11	17	17	10	9	4			86,1

Golden Rain has 96,4 %, Victory 95,5 % and Bell II 91,8 % of seed setting.
The difference between selected and non-selected plants gives $t=5{,}86$ and $P<1/1000$.

III. CYTOLOGICAL ANALYSIS OF MEIOSIS.

For further investigation of this partial sterility it was near at hand to study the meiosis of F_1 plants of crosses between these black and white oat types. In the summer of 1950, thus, crosses between black and white oats were carried out on a large scale by Dr. BERNSTRÖM. He, however, got no time to complete the investigation. The following varieties were used as parents in the crosses:

Black.

Roslags (indigenous variety),
Fyris (strain selected from the above),
Great Mogul II (from the cross Bell II × Great Mogul I),
Visingsö (indigenous variety).

White.

Blenda (from the cross Star × Eagle),
Eagle (from the cross v. Lochows Yellow oat × Victory),
Sv 01583 b (from the cross Victory × an indigenous variety).

Seed setting after crossing was, as usual for oats, not very good. Altogether we obtained 63 F_1 grains. These were sown spaced in the field and so was the corresponding parental material for comparison. Due to the spaced stand the plants had a very good tillering and were well developed but rather late in shooting and ripening.

Panicles with pollen mother cells (P. M. C.) in stages of meiosis were fixed from every F_1 plant and from a corresponding number of parental plants. Fixation was made in Carnoy for 2 hours and after that the panicles were kept in 70 % alcohol before the work could be carried on. The glumes were then removed and the flowers with the now free anthers were washed in distilled water. They were treated for 5—6 min. with n-HCl at 60° C, were washed and moved into Feulgen stain. P. M. C. were studied in squash preparations, which were made permanent by embedding in euparal. The meiosis stages from metaphase I to the tetrads were studied. Samples of pollen in acetocarmine-glycerine preparations were studied to get informations about the pollen quality.

1. MEIOSIS IN PARENTAL MATERIAL.

In the present material of black and white oat varieties the study of meiosis has mainly been carried out at metaphase I. In some P. M. C. univalents were found. These are the most common type of irregularities

observed. They were found in 2,7 % of the P. M. C. studied. In 5 P. M. C. out of 700 studied, or in 0,7 %, we observed multivalents. This figure is a minimum value, since as a rule we have not been able to observe zigzag orientated multivalents, but only such as have formed open rings or chains. On the other hand, these of the latter types more often result in an irregular progeny (*cf.* HAGBERG, 1954). These »normally occurring meiotic irregularities» are certainly mainly caused by pairing between semihomologous chromosomes, belonging to different genomes of the allohexaploid oats.

In the first anaphase of meiosis we have found lagging univalents in 2,4% of the P. M. C. These univalents are auto-orientated in the equatorial plane of the P. M. C. and are divided, each chromatid going to each of the two anaphase groups. As a rule, these »univalent chromatids» are enclosed in the telophase and interkinesis nuclei. At second anaphase these chromatids are not able to divide once more normally (misdivision may happen, as will be later shown and discussed). The chromatids are lagging and will be eliminated in many cases, forming micronuclei in the tetrads. One single case of a bridge at late first anaphase has been observed and it probably represents a rare occurrence in the parental material.

As to differences between different parental lines in the frequency of irregularities, a slight tendency is to be observed. A »pure line» from an indigenous variety such as Visingsö is quite without irregularities in meiosis. The high-bred varieties from crosses, *e. g.* Blenda and Great Mogul II, have a higher degree of meiotic irregularities. They have not more than about 5 % of P. M. C. with irregularities, however. The parental material here investigated is too small to give significance to this tendency.

2. MEIOSIS IN THE F_1 GENERATION OF CROSSES BETWEEN BLACK AND WHITE OATS.

Table 2 shows that the pollen quality of F_1 plants is slightly disturbed as compared with that of the parental material. There is, however, not a marked decrease of pollen fertility, but just a slight one. In studying meiosis, however, we found that at first metaphase there was a high frequency of irregularities. More than half of the P. M. C., or about 56 % of them, contained either univalents or multivalents or both types of disturbances. In some cells more than one multivalent were observed. The difference in frequency of irregularities between

TABLE 2. *Pollen fertility in crosses between black and white oat varieties.*

(*a*) *Parental and F_1 generations:*

	Per cent of good pollen															
Generation	62	64 . . . 74	76	78	80	82	84	86	88	90	92	94	96	98	100 M
F_1		1		1	2			1	3	3	2	2	8	5	6	89,8
P									1		7	5	13	40	16	96,2

The difference between F_1 and P generation gives $t = 6{,}87$ and $P < 1/1000$.

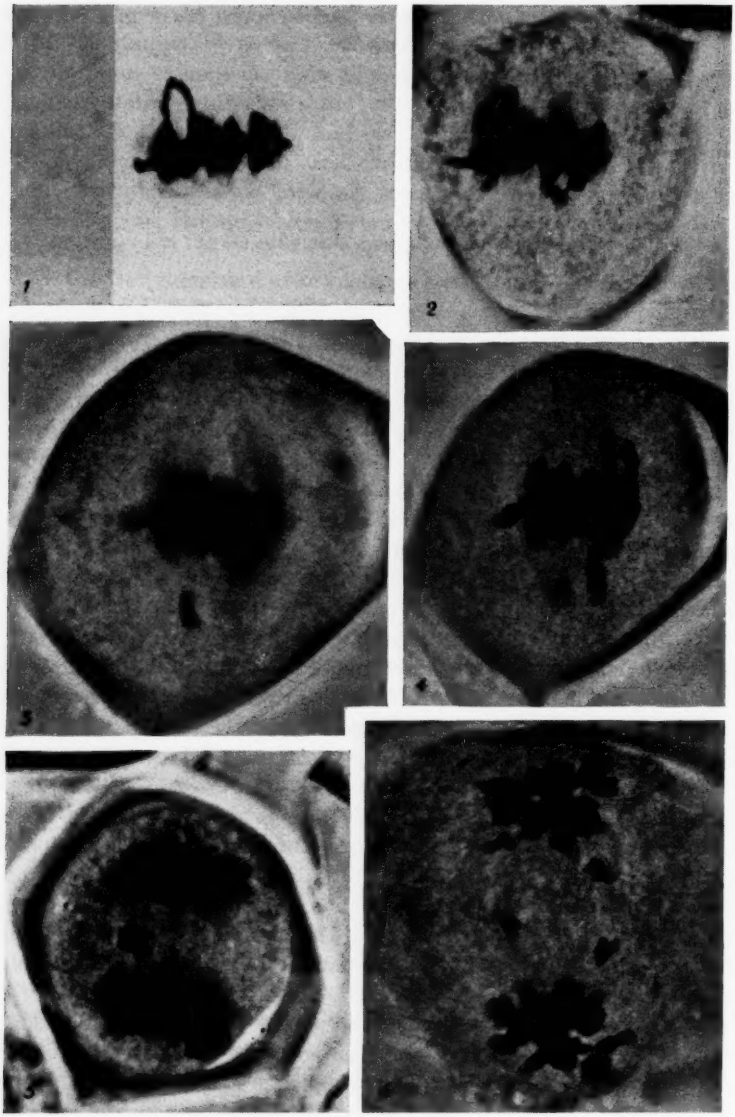
(*b*) *Late generation:*

	Per cent of good pollen														
	87	88	89	90	91	92	93	94	95	96	97	98	99	100 M
Fert.-sel.				1	2	4	5	12	19	25	19	6	6	96,1
Ster.-sel.	1	1		1	1	6	4	5	11	15	13	6	5	95,9
P-generation	1			4	3	2	3	5	8	21	19	10	6	96,2

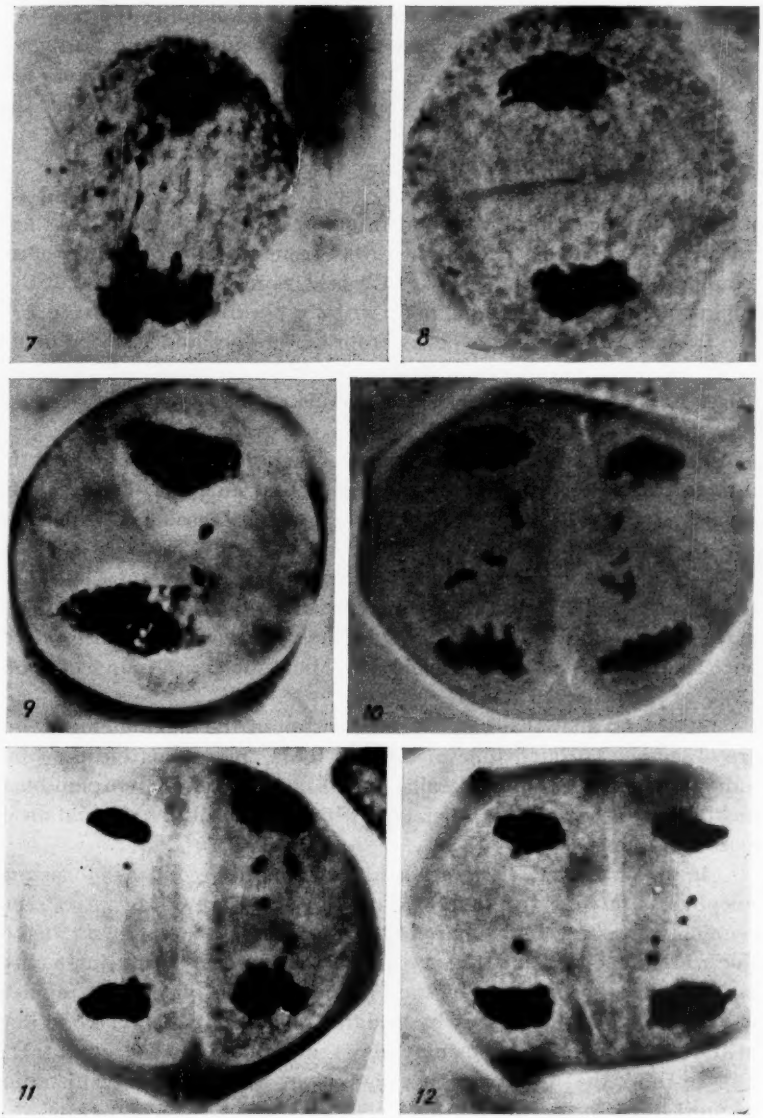
the parental material and the F_1 is significant, $\chi^2 = 504$ and P much less than 0,001. The figure of the frequency of multivalents is a minimum as pointed out earlier; we were not able to detect all zigzag orientated tetravalents.

Through studies of later meiotic stages we have possibilities to follow what happens in these irregular P. M. C. At first anaphase some univalents and some not co-orientated chromosomes, which were involved in tetravalents (*cf.* HAGBERG, 1954), will be lagging at the equatorial plane (Fig. 5). About 45 % of the P. M. C. were found to contain such laggards at this stage. The centromere in such a lagging chromosome will be auto-orientated and is divided as in a normal mitosis (Fig. 6). As a rule, the daughter chromatids reach the telophase groups in due time to get into the interkinesis nuclei. Sometimes, however, we found micronuclei in the dyads (Fig. 10).

In about 15 % of the P. M. C. at first anaphase bridges are found. In a few cases we also found fragments (Figs. 7 and 8) but very often fragments are lacking (*cf.* MAC KEY, 1954, Figs. 5 and 6). A bridge without fragment is also found in the parental material. It looks, however, as if the bridges in the F_1 material are more tough; terminalization is delayed. In the F_1 plants the bridges from the first anaphase sometimes are still remaining during the second division. Most often, however, the bridge is broken or the chromatids are successfully separated without breakage before a cell wall is formed in the dyad.



Figs. 1—6. First metaphase and anaphase of meiosis of the F_1 generation of crosses between different varieties of black and white oats. More than 50 % of the P. M. C. contain univalents, multivalents or both types of irregularities.



Figs. 7—12. Later stages of meiosis in the same material. For discussion and explanation of the figures, see text.

Bridges without fragments are with great probability the result of pairing between two not fully homologous chromosomes (*cf.* HAGBERG, 1954, Fig. 13). The difficulties in terminalization of the chiasmata are caused by and depending upon the degree of differences between the non-homologous segments (*cf.* MAC KEY, 1954, for wheat material and HAGBERG, 1954, for barley).

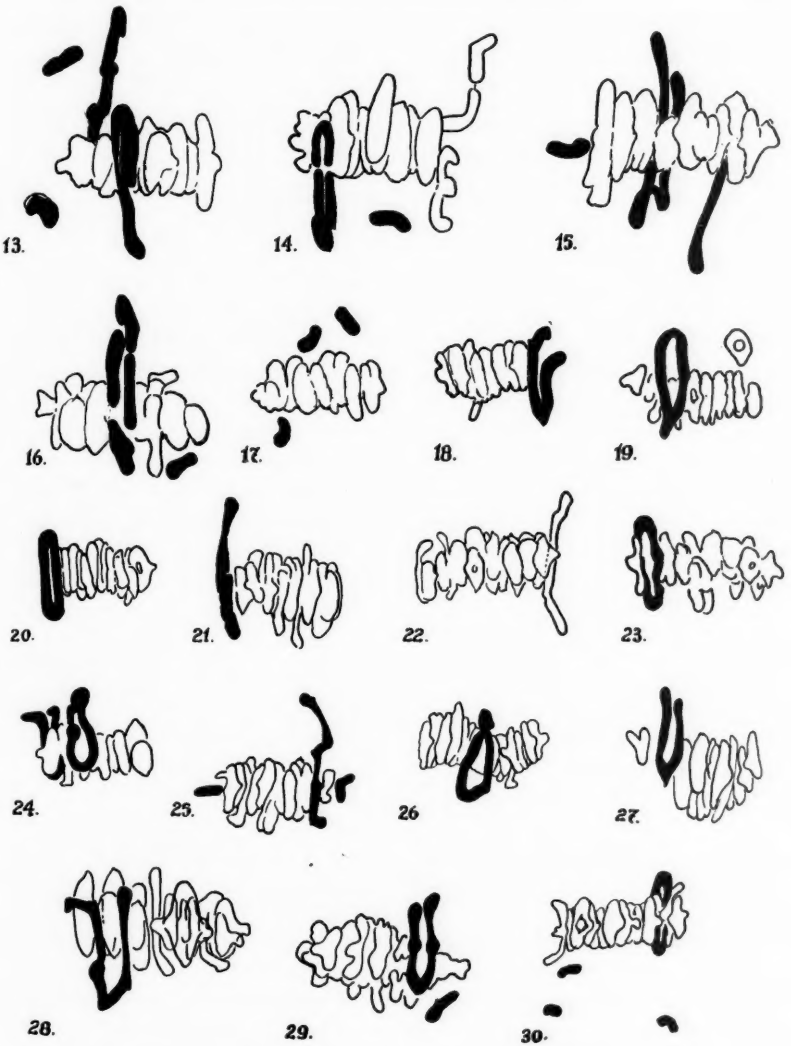
It is rather probable that most cases of bridges in the material are of this type. Some times we found fragments without bridges and these fragments seem to be chromatin drops from already separated chromosomes. Fragments in P. M. C. with bridges may very well be of this origin and, thus, have nothing to do with the coexistent bridges (Fig. 39). The fragments are often far away from the bridges and seem no doubt to be drops of chromatin pulled off already terminalized »bridges» (Figs. 7 and 8). Probably they are eliminated and not included in the interkinesis nuclei.

At second anaphase and telophase of meiosis we often observed the chromatids from chromosomes divided at first anaphase laying at the equatorial plane between the two groups of chromosomes, which are already normally divided (Figs. 10, 11, 12, 41 and 42). About half the number of P. M. C. (51 %) shows such lagging chromatids at second anaphase. Some of these chromatids reach the poles and are included in the nuclei. Many of them are not included, however, but form micronuclei in the tetrads (Figs. 44 and 45). Micronuclei are found in about 43 % of the observed tetrads.

At second anaphase cases of misdivision of the lagging chromatids are observed. Through this misdivision these chromatids escape elimination; and in the pollen grains formed there will be chromosomes lacking one arm and the other arm will be doubled, *i. e.* formation of isochromosomes.

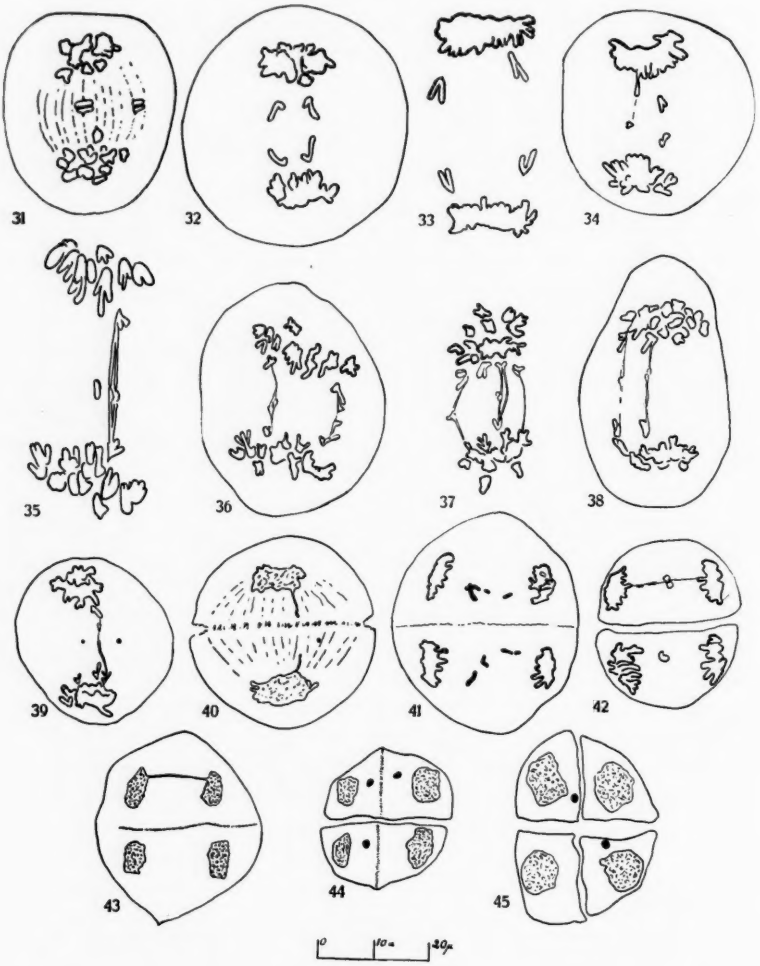
In a few P. M. C. there are bridges found at second anaphase and telophase also (Figs. 42 and 43). Probably these are the result of sister reunion in broken, double chromatid bridges at first anaphase. Most of the bridges at first anaphase and telophase, however, were single chromatid bridges; a breakage of such a bridge cannot give sister reunion. This is probably the reason why bridges are less common at second than at first anaphase. Inversions may play some rôle as the cause of bridges but we think that the common dishomology is valid as an explanation of most of the bridges.

The meiotic irregularities described above have occurred in about the same frequencies in all the tested cross combinations between black



Figs. 13—30. Metaphase of meiosis of the F_1 generation of crosses between different varieties of black and white oats. *Cf.* Figs. 1—4.

and white oats. Significant differences have not been observed in the limited material investigated. The important result is, however, the



Figs. 31—45. Later stages of meiosis in the same material. *Cf.* Figs. 5—12.

significant difference of meiotic irregularities between parental line material on one side and the F_1 material of the cross black × white on the other. The fertility, pollen quality, as well as seed setting, is slightly decreased (for decrease in pollen quality, see Table 2, $t=6{,}87$ and $P < 0{,}001$). This decrease is, however, not reflecting the high degree of

meiotic disturbances. These may result in gametes with abnormal genotypes which, however, are not lethal due to the allohexaploidy of the oat.

3. MEIOSIS IN PLANTS OF LATER GENERATIONS OF THE CROSS BLACK × WHITE OATS.

An older material has been grown at the same time and on the same field, and in some plants meiosis has been studied. These plants were divided into two groups: one selected for high fertility and one for low. Pollen fertility was determined in both groups, altogether 168 plants were studied. There was, however, several generations after crossing no difference between the groups and no difference between the parental material and the cross-progenies (see Table 2 *b*).

Meiosis was studied in 11 plants. One of them showed irregularities at first metaphase in 20 % of the P. M. C. and in two other plants there were found irregularities in 6, respectively 7 % of P. M. C. studied. The remaining eight plants had the same frequency of disturbances as observed in the parental material. In later meiotic stages we also found in the three irregular plants a somewhat higher frequency of disturbances — laggards and chromatid bridges — than normally observed. The number of chromosomes was, unfortunately, not estimated in the plants. In the F_2 generation there will certainly occur a variation in chromosome number and in structure of the individual chromosomes causing complications at meiosis in many of these F_2 plants. This will be more thoroughly investigated and described in another paper.

IV. DISCUSSION.

In allopolyploid plants, as for instance oats, pairing between not fully homologous chromosomes sometimes will occur normally. The degree of this pairing depends upon how related the different genomes included are. This type of pairing will result in multivalents and sometimes in univalents. Such irregularities are also found to some extent in the oat varieties studied, the black as well as the white ones. Meiotic irregularities, however, seem to be more common in cross-bred strains than in »pure line» material. Probably, as a rule, there are some smaller structural differences between the lines selected from crosses. GUSTAFSSON (1947) has shown that there is a tendency to higher frequency of mutations in varieties which originate from crosses than in »pure line» varieties.

In the present material we found a frequency of P. M. C. with meiotic irregularities at first metaphase of about 3 %. HOWARD (1948) has described meiosis in some oat varieties, used in certain crosses. For the three varieties investigated he found 0, 1,5 and 4,8 %, respectively. MAC KEY (1954) has given a summarizing review of corresponding investigations of wheat varieties. Much more such studies are made on wheat than on oats. Both crops are, however, self-fertilized, allohexaploid species and since long time cultivated. The meiotic conditions seem to be very much the same in both these species. Thus, MAC KEY (*l. c.*) for different varieties of wheat stated that the frequency of P.M.C. with meiotic irregularities varies from 0,6 to 10 %. In his own material, the winter wheat variety Skandia III, MAC KEY found 4,2% of the P. M. C. with irregularities in meiosis. Multivalents are formed by pairing of semihomologous chromosomes and single univalents may also be a result of this mode of pairing. The present oat material behaves just as the wheat material and we found a slightly higher frequency of meiotic disturbances than did HOWARD. He has, however, mainly studied anaphase I and later stages and that will probably be the cause of the difference.

HOWARD (1947) has also studied crosses between closely related oat varieties. In the F_1 material he found a slightly higher frequency of meiotic disturbances than in the pure parental strains. JOSHI (1951) has got the same result: 3,6 % in crosses and less than 2 % in the parental lines. The frequency is, however, of about the same order of size as found in the parental material. On the other hand, when HOWARD (*l. c.*) crossed as far differentiated parental varieties as a winter oat and a spring oat, he found in the F_1 plants a much higher frequency of meiotic irregularities. This frequency varied from 17 to 28 % in his material. JOSHI (1951) found in the same crosses 14—31 %. In the crosses between black and white oat varieties we found as an average 56 % of the P. M. C. with observable meiotic irregularities. This cannot be caused by semihomologous pairing only but is probably caused by a structural differentiation which is of the same order as the interspecific differentiation between *Avena* species with 42 chromosomes (JOSHI, 1951). Without giving any detailed data, MAC KEY (1954) states from experiences of his wheat material that »hybridization and especially crosses between widely separated varieties considerably increase the frequency of irregularities».

In another paper HOWARD (1948) studied different plants of the variety Picton, which was bred from a cross between winter and spring

oats. He found great differences in the meiotic behaviour of the different plants. Some plants had about 50 % of the P. M. C. irregular and some single plants had each P. M. C. irregular. As an average, however, the Picton plants had about 4 % of their P. M. C. irregular. The progeny of an irregular Picton plant was studied by JOSHI as to the somatic chromosome numbers and the meiotic behaviour. Most of the plants were aneuploids and their meiosis was, of course, not quite regular.

These results are in good agreement with the experience from the breeding work with black×white oat crosses and with the results obtained from cytological studies of this cross. There is certainly a differentiation in chromosome structure between these far related oat varieties. This may also be in good agreement with FRÖIER's (1946) statement that there are essential genotypic differences between black and white oats as to the chlorophyll factors. In polyploid self-fertilizers this differentiation in chromosome structure can occur to an extent which often is not possible in a cross-fertilizer or in a diploid crop. We may here only cite MÜNTZING's (1938) thorough investigations of the allotetraploid species *Galeopsis Tetrahit*.

SUMMARY.

Since a long time it has been a desire to combine the habit of growth of the black oats of central Sweden with the grain quality and other valuable characters of the Probstei oat type grown in southern Sweden. The crossed material has, however, shown a partial sterility and the breeding work has not been fully successful. A cytological analysis of the meiosis of F_1 plants shows that more than half of the pollen mother cells are irregular in some way or other. The different types of meiotic irregularities observed are described and discussed.

LITERATURE CITED.

1. ATTERBERG, A. 1889. Skandinaviens och Finlands hafrevarieteter, deras kännedom och utbredning. — Tioårsber. Kalmar Kem. Stat. 1877—1887, Bil. 1.
2. FRÖIER, K. 1946. Genetical studies on the chlorophyll apparatus in oats and wheat. — Hereditas XXXII: 297—406.
3. GUSTAFSSON, Å. 1947. Mutations in agricultural plants. — Hereditas XXXIII: 1—100.
4. HAGBERG, A. 1954. Cytogenetic analysis of erectoides mutations in barley. — Acta Agr. Scand. IV: 472—490.

5. HOWARD, H. W. 1947. Meiotic irregularities in hexaploid oats. I. Univalent frequencies in spring×winter variety hybrids of *Avena sativa*. — Journ. Agr. Sci. 37: 139—144.
6. — 1948. Meiotic irregularities in hexaploid oats. II. A cytological survey of the variety Picton. — Journ. Agr. Sci. 38: 332—338.
7. JOSHI, A. B. 1951. Cytogenetics of hexaploid oats, with reference to the occurrence of off-types in new varieties, and the origin of *Avena sativa* L. — Abstr. Diss. Univ. Cambr. 1949—50: 22—23.
8. MAC KEY, J. 1954. Neutron and X-ray experiments in wheat and a revision of the speltoid problem. — Hereditas XL: 65—180.
8. MUNDT-PETERSEN, K. 1937. Havreodlingen i Sverige i dess beroende av klimatiska faktorer. — Sveriges Utsädesförenings tidskr., 47: 389—415.
10. MÜNTZING, A. 1938. Sterility and chromosome pairing in intraspecific *Galeopsis* hybrids. — Hereditas XXIV: 117—188.
11. NILSSON, HJ. 1903. Svalöfs Guldregnshafre. — Allm. Svenska Utsädes A.B. Årsredogörelse 1903: 13—18.
12. NILSSON-EHLE, H. 1906. Kort sammanställning af resultaten från Utsädesföreningens jämförande försök med olika hafresorter 1893—1905. — Sveriges Utsädesförenings tidskr., 16: 45—78.
13. — 1908. Svalöfs Segerhafre (0355). — Allm. Svenska Utsädes A.B. Årsredogörelse 1908: 8—11.
14. TORSSELL, R. och MAC KEY, J. 1947. Förädling av mellansvensk svarthavre vid Sveriges Utsädesförening. — Sveriges Utsädesförenings tidskr., 57: 163—178.
15. ÅKERMAN, Å. 1951 a. Svensk växtförädling, del I, Åkerbruksväxterna. — Stockholm, Natur och Kultur, 139—168.
16. — 1951 b. Några erfarenheter rörande angrepp av fritflugelarver på havre. — Sveriges Utsädesförening tidskr., 61: 108—118.

CONTENTS.

I. Introduction 438
II. The fertility in two white×black oat crosses 439
III. Cytological analysis of meiosis 441
1. Meiosis in parental material 441
2. Meiosis in the F_1 generation of crosses between black and white oats .. 442
3. Meiosis in plants of later generations of the cross black×white oats .. 449
IV. Discussion 449
Summary 451
Literature cited 451

Communications from the Swedish Seed Association. No. 84.

HYBRIDIZATION OF WHEAT AND RYE AFTER EMBRYO TRANSPLANTATION

BY *OVE L. HALL*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

I. INTRODUCTION.

FOR several years investigations on hybrids between wheat and rye have been made at the Institute of Genetics at Lund (*cf.* MÜNTZING, 1939, 1943, 1948). In this connection a paper by PISSAREV and VINOGRADOVA (1944), dealing with the effect of embryo transplantation on the crossability of wheat and rye, is of special interest.

During their hybridization work, PISSAREV and VINOGRADOVA observed that the embryos and endosperm began to develop after pollination of the wheat flowers by rye pollen, but that in most cases further growth of the organs soon ceased completely. They consider that this disturbance partly depends on biochemical differences between the components used in the crosses and that this barrier may be overcome by embryo transplantation. By this technique it should be possible to modify the components used, with increased ability of hybridization as a result. Their arguments are strengthened by results gained by SCHANDER on the part played by the endosperm in the development of the plant (SCHANDER, 1934).

PISSAREV has worked out a technique for transplantation, described as follows: »The embryo together with the scutellum was cut from the dry caryopsis with the help of a safety-razor blade; care was taken to cut as little endosperm as possible. The embryo was then transferred on to the 'endosperm wilding' whose embryo had preliminarily been cut out. The graft embryo was fixed in place by means of a paste prepared from a flour of the same genus to which the 'endosperm wilding' belonged».

According to PISSAREV and VINOGRADOVA, the wheat plant growing from the rye endosperm did not differ in morphological respects from the controls, but on the other hand there was a chemical difference. It was also apparent that the wheat transplanted on to the rye endosperm had a higher degree of crossability with rye compared with ordinary wheat. Three different varieties of wheat were investigated, Lutescens 62, Hybrid 170 and Aurora. The results of the crosses are summarized in the table below.

Percentage of successful crosses.

Variety	Control group	Grafted group
Lutescens 62	4,3	25
Hybrid 170	3,4	11,5
Aurora	2,8	19,2

This surprising result led to the present investigation carried out with Swedish material.

II. EXPERIMENTAL WORK.

A preliminary experiment was started in the spring of 1952 when, during the last week of April, 200 transplantations of wheat embryos on to rye endosperm were carried out. The spring wheat variety »Ella» and the spring rye variety »Od» were the material used. The methodical instructions given by PISSAREV and VINOGRADOVA for the transplantation of embryos were followed exactly. About an hour after the wheat embryo had been transferred to the rye endosperm, the »graft-kernel» was placed in sterilized soil in small pots. At the same time 200 untreated wheat kernels were also planted as control material. When the plants had reached a height of about 15 cms, they were moved from the greenhouse into outdoor frames. At the end of June 1005 flowers were emasculated in the transplanted group, as well as 206 flowers in the control group; the ears were isolated in pergamyn bags. The emasculated wheat flowers were pollinated with rye pollen after a week. About six weeks later the kernels produced were harvested. In the transplanted group, 17 hybrid kernels had been formed by the 1005 flowers pollinated, while on the other hand no kernels had been formed by the 206 pollinated flowers in the control group. The considerable difference between the number of flowers treated in the two groups was due to a long period of bad weather that hindered work, and also to an irregular supply of rye pollen.

It should be mentioned that both this year's emasculating and pollination work and next year's, which will be described below, was carried out by experienced personnel accustomed to such work, but without knowledge of the aim of the experiment.

On the basis of the above figures it was obviously impossible to draw definite conclusions as to the effect of embryo transplantation on the crossability of the two varieties used. There was an indication of a positive result, however, which stimulated to further experiments.

At the beginning of April, 1953, a new experiment was thus started, this time on a larger scale. 500 transplantations of wheat embryos on to rye endosperm were then made. This group will from now on be called the V/R group. In addition, the same amount of transplantations of wheat embryos were made on to another wheat endosperm of the same variety. The latter group formed the control material and is called V/V below. As in the previous year's experiment, the transplantations were carried out according to PISSAREV and VINOGRADOVA's instructions, and Ella spring wheat and Od spring rye were used this time, too.

The »graft-kernels» were planted in sterilized soil in small pots, placed in a greenhouse at a temperature of 20° C. 500 untreated wheat kernels were now also planted to determine the vitality of the sowing, as well as 200 detached embryos. The purpose of doing this was both to decide whether the detached embryos could be developed without their endosperm, and to what extent the embryos made use of the endosperm on to which they had been transplanted.

After about three weeks the material was moved from the greenhouse and put in frames. It was placed in groups with the four abovementioned groups side by side. With the exception of the plants that had their endosperm removed, the plants showed normal speed of growth and there was not any noticeable difference between the groups. The group with the detached embryos was even at an early stage less developed and had a lower speed of growth compared with the three other groups. In the middle of June when the plants were ready to be emasculated, there were 276 plants with ears in the V/R group and 417 plants with ears in the V/V group. 438 plants with ears had grown from the untreated wheat kernels and 27 from the detached embryos. 87 % of the untreated kernels had thus produced plants with ears, 83 and 55 being the corresponding percentages for the V/V and V/R groups. Only 13 % of the detached embryos had germinated and grown into mature plants. It can at once be seen from the figures that there is a highly pronounced difference in the ability of the embryos to be developed if they have been detached, or if there has been an available endosperm they could utilize. The embryos transplanted to another wheat endosperm develop six times more often into plants than the detached embryos. The wheat embryos transferred to rye endosperm grow into plants with ears four times more frequently than the wheat embryos detached from their endosperm.

There is also a significant difference between the V/V and V/R

categories. The percentage values 83 and 55 were obtained from the absolute ratios 417 : 83 and 276 : 224. A χ^2 test was made giving a χ^2 of 93 and a P smaller than 0,001.

III. POLLINATION RESULTS.

2813 flowers from 101 plants in the V/V group and 2897 flowers from 102 plants in the V/R group were emasculated during the time from the 18th to the 20th of June. The ears were isolated after emasculation in pergamyn bags. After about a week the emasculated flowers were pollinated with rye pollen and the ears again isolated.

About the end of July and the beginning of August the experimental plots were harvested and the amount of kernels obtained after pollination was counted for each ear. It turned out that there were in all 75 hybrid kernels in the V/V group, *i. e.* the control group, while there were 400 in the V/R group, *i. e.* the experimental group. The figures for the successful crosses were 2,6 % for the control material and 13,8 % for the V/R group. *Thus, the wheat transplanted on to rye endosperm formed about five times as many hybrid kernels as the wheat transplanted on to wheat endosperm.*

The significance of the difference in the amount of kernels obtained between the groups V/V and V/R can be verified according to the χ^2 method below:

	Number of unsuccessful crosses	Number of successful crosses	n
V/V	2738	75	2813
V/R	2497	400	2897
Total	5235	475	5710

A comparison between the above ratios gave a χ^2 value of 230 which for 1 degree of freedom corresponds to a P-value much smaller than 0,001. As the two groups had been treated in the same way and grown up under similar conditions, the conclusion must be drawn that the factor that was different in the groups also gave rise to the difference in crossability. This factor has been the endosperm on which the respective embryos have been developed.

The results of the experiment described up to now can be summarized in the following table:

Group:	V/V	V/R	Untreated wheat	Detached embryos
Number of kernels planted	500	500	500	200
Number of plants with ears	417	276	438	27
Percentage of plants with ears ..	83	55	87	13
Number of pollinated flowers	2813	2897	—	—
Number of hybrid kernels	75	400	—	—
Percentage of hybrid kernels ...	2,6	13,8	—	—

How the hybrid kernels were distributed among the different plants is apparent from the columns below:

Number of kernels	Number of plants		Number of kernels	Number of plants	
	V/V	V/R		V/V	V/R
0	60	28	12	0	3
1	18	18	13	0	1
2	11	14	14	0	1
3	9	10	15	0	3
4	3	3	16	0	1
5	0	5	17	0	0
6	0	2	18	0	1
7	0	0	19	0	1
8	0	4	20	0	0
9	0	3	21	0	0
10	0	1	22	0	1
11	0	2			

Evidently 60 plants in the V/V group were completely without hybrid kernels, while only 28 plants in the V/R group had not formed any kernels. The maximum formed has been 4 kernels per ear in the control material, but an ear with not less than 22 hybrid kernels was found in the V/R group.

From what has been said above, it is clear that part of the result that PISSAREV and VINOGRADOVA reported in their work quoted above, could be reproduced. It has been shown with great statistical certainty that the crossability between Ella spring wheat and Od spring rye can be increased if the wheat plants used for the crosses are derived from embryos which were transplanted on to rye endosperm.

Also this year (1954) similar experiments are under way with kernels obtained from plants used in 1953 as material. Last year's hybrid kernels have been planted; the plants obtained from them will be studied.

Biochemical analyses of the material used is also being carried out parallel with this work. This is primarily aiming at a comparison of

the composition of the aminoacids and carbohydrates in the different cereals used. Varieties of wheat and rye, other than those mentioned above, will also be studied.

June, 1954.

LITERATURE CITED.

1. MÜNTZING, A. 1939. Studies on the properties and the ways of production of rye-wheat amphidiploids. — Hereditas XXV, pp. 387—430.
2. — 1943. Några försöksresultat med rågvete och tetraploidt korn. — Nordisk Jordbrugsforskning. Hefte 5—6, pp. 250—262.
3. — 1948. Några data från förädlingsarbetet med tetraploid råg och rågvete. — Nordisk Jordbrugsforskning. Hefte 1—3, pp. 490—507.
4. PISSAREV, W. E. and VINOGRADOVA, N. M. 1944. Hybrids between wheat and *Elymus*. — Comptes Rendus (Doklady) de l'Acad. des Sciences de l'URSS. Vol. XLV, No. 3.
5. SCHANDER, H. 1934. Keimungsphysiologische Studien über die Bedeutung der Aleuronschicht bei *Oryza* und anderen Gramineen. — Zschr. f. Botanik 27, pp. 433—515.

THE CYTOLOGICAL BASIS OF POLYMORPHISM IN POA ALPINA

BY *ARNE MÜNTZING*
INSTITUTE OF GENETICS, LUND, SWEDEN

I. INTRODUCTION.

IN the large grass genus *Poa* the basic chromosome number is known to be 7, but in some of the apomictic species aneuploidy prevails and at the same time there is a marked intraspecific variation in chromosome number. Thus, for instance, in *Poa pratensis* the majority of the apomictic biotypes have numbers ranging from 50 to 100 (*cf.* MÜNTZING, 1932; ÅKERBERG, 1941; HARTUNG, 1946), whereas in *Poa alpina* most of the Scandinavian and Swiss apomicts have numbers between 25 and 50 (*cf.* MÜNTZING, 1932, 1940).

Poa alpina is frequent in North Sweden, where it is met with not only in the alpine and subalpine regions, but also in the lowland. The species also occurs in some regions in South Sweden, presumably as a glacial relic, and this is especially true of the two large islands Öland and Gotland in the Baltic Sea off the Southeastern part of the country. *Poa alpina* is also known to occur in some mountains in the province of Västergötland in Southwest Sweden.

In the papers cited above preliminary data on the chromosome variation of the species have been given, and with regard to the Swedish material it was found to be especially interesting that the same special aneuploid chromosome number, 33, was found to be present in the material from Västergötland and Gotland and also in some of the North Swedish biotypes. Other North Swedish biotypes had $2n=38$ and a strain from Öland had $2n=35$. This preliminary information stimulated a more thorough investigation, the results of which are given in the present paper.

In 1942 a collecting tour was undertaken in North Sweden, especially in the province of Lapland, but seed samples were also gathered in the surrounding provinces of Jämtland, Norrbotten and Ångermanland. At higher altitudes no ripe seeds could be obtained and in such cases living plants were collected and sent to Svalöf for further examination.

Material from Öland and Gotland was collected in 1944. The former

island was visited rather early in the season and therefore living plants had to be taken, no seeds being ripe. The other island, Gotland, was visited somewhat later and in this case seed material could be obtained. Additional material from Sweden, Norway, Iceland and Greenland was obtained thanks to the courtesy of several colleagues. My sincere thanks for this valuable collaboration are due to Doctors C. L. KIELLANDER, J. A. NANNFELDT, S. ERLANDSSON, G. ÖSTERGREN, N. ALBERTSSON, A. LÖVE, M. WESTERGAARD, C. A. JÖRGENSEN, and E. RUNQUIST. During my own collecting trips Mrs. G. MÜNTZING functioned as a sharp-sighted observer who discovered many biotypes which would otherwise have been overlooked.

The seed material collected in North Sweden was germinated in the spring of 1943 and the full-grown plants studied in the summer of 1944. Some of the plants collected in North Sweden in 1942 produced panicles and ripe seeds in the same year after transplantation to Svalöf. Progenies of these plants were included in the material germinated in the spring of 1943. Other transplanted plants did not produce seeds until 1943, and in these cases progenies were raised in the spring of 1945. At the same time the material from Öland and Gotland was germinated as well as new progenies from the North Swedish material studied in 1944. The Gotland material was raised from panicles collected in the natural habitats, the Öland material from panicles produced at Svalöf in the year of transplantation. With few exceptions the progenies were raised from seeds of a single openpollinated panicle, each progeny consisting of 16 plants or a slightly lower number, owing to mortality in the field.

As a rule, root-tip fixations were made of three plants per progeny and also, before that, of all the plants collected in the field and sent to Svalöf. The root tips were fixed in diluted chrome-acetic-formalin, the plants before fixation being kept over-night at low temperature (slightly above zero) to bring about chromosome contraction which greatly facilitates the counts. The root tips were sectioned and stained with crystal violet. My sincere thanks are due to Miss. M. PALM and Miss J. WARDEN for valuable help with the somatic chromosome counts.

II. CHROMOSOMAL VARIATION.

A summary of all chromosome numbers determined in the seed-producing biotypes is given in Table 1. With certain exceptions, to be discussed below, this table roughly expresses the chromosomal variation

TABLE 1. *Somatic chromosome numbers in material from different regions.*

Chromosome number	North Sweden			Gotland			Öland			Norway			Iceland			Greenland		
	Accurate	±	Total	Accurate	±	Total	Accurate	±	Total	Accurate	±	Total	Accurate	±	Total	Accurate	±	Total
26		1	1															
⋮		—	—															
32	1	1	2							2	4	6						
33	116	10	126	44		44				12	4	16	4		4			
34	—	1	1	5		5				—	1	1	10	1	11			
35	3	2	5	—	1	1	7	3	10	—	—	—	—	1	1			
36	9	1	10	—	—	—	—	—	—	—	—	—	—	—	—			
37	14	9	23	1	—	1	—	—	—	5	—	5	—	—	—			
38	175	22	197	—	—	—	—	—	—	1	2	3	1	—	1			
39	37	9	46	—	—	—	1	—	1	—	—	—	1	—	1		1	1
40	12	1	13	6	—	6	11	2	13	—	—	—		1	1		—	—
41	1	—	1	72	1	73	102	10	112	—	—	—		—	—		—	—
42	10	2	12	1		1	1	—	1	—	1	1		—	—	2	2	4
43	7	3	10	3		3	5	—	5	—	3	3		—	—		4	4
44	49	5	54	3		3		—	—	—		—		1	1		1	1
45	10	3	13	3		3		—	—	—		—					3	3
46	—	—	—	—		—		—	—	—		—					—	—
47	—	1	1	—		—		—	—	—		—					—	—
48	1	1	2	—		—		—	—	—		—					1	1
49	1	—	1	—		—		—	—	—		—						
50	3	1	4	—		—		1	1	—		—						
51				—		—		—	—	—		—						
52				—		—		—	—	1		1						
⋮				—		—		—	—									
60				1		1		—	—									
61								2	2									
Total	449	73	522	140	2	142	127	18	145	21	15	36	16	4	20	2	12	14

in the different regions studied. The values given are differentiated into accurate and ± counts, the former being represented by three different somatic plates giving the same value, the latter ones by cases in which fixation was less satisfactory, the plates giving slightly different values. The relative frequencies of accurate and ± counts differed in the various groups, the Gotland material giving the best results and the Greenland material the worst.

Starting with the *North Swedish material* (chiefly from Lapland) we find that 18 or 19 different chromosome numbers are represented, ranging from 32 (or possibly ±26) to 50. The predominating numbers

are 38 and 33 and a third maximum is present at 44. Many plants were also found to have 39 chromosomes, whereas the other numbers are relatively rare. Thus, there is certainly no random distribution of the chromosome numbers but rather a few very pronounced peaks.

The *Gotland material* is rather interesting by showing two very clear maxima, one at 41 and the other one at 33. Thus, one of the maxima is represented by the same chromosome number as in one of the predominating maxima of the North Swedish material, whereas the other and predominating peak is quite new, the number 41 being represented by more than half of the Gotland material but only by a single plant in the material from North Sweden.

The counts from the *Öland material* revealed one important similarity to and one important difference from the Gotland material, the number 41 being quite predominating in Öland and the number 33 quite absent. Instead, there is a small group with 35 chromosomes and a few other chromosome numbers.

The small material from *Norway* indicates a similar chromosomal variation as in North Sweden, the numbers found ranging from 32 to 52 with one rather clear maximum at 33. The material from *Iceland* and *Greenland* shows a variation from 33 to 48 and is further discussed on pp. 489—491.

TABLE 2. *Chromosome numbers in viviparous plants from North Sweden.*

	Chromosome number:																		n
	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	
Accurate values		3	—	—	1	—	—	—	10	5	—	—	—	—	—	—	1	—	8	28
± »	1	3	3	—	—	—	5	2	6	7	1	—	—	1	—	—	—	2	8	39
Total	1	6	3	—	1	—	5	2	16	12	1	—	—	1	—	—	1	2	16	67

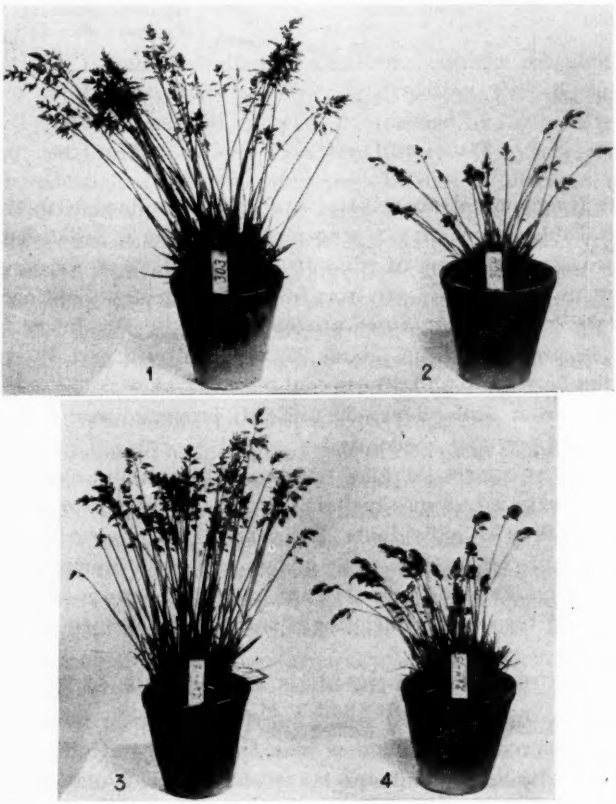
A special category is represented by *viviparous plants* from North Sweden, in which the above result was obtained (Table 2). A total of 67 plants belonging to 18 different progenies were examined. The fixations were not very satisfactory and therefore the majority of the counts are included in the ± category. Nevertheless, a series of different chromosome numbers are represented and among these some (54, 55, 56) that are higher than those found in the seed-forming plants from the same regions. On an average the chromosome numbers of the viviparous plants show a range (38 to 56) which seems to be higher

than that found in the seed-forming plants (26 to 50). This will be further discussed below in connection with the analyses of the frequency and distribution of the different biotypes (pp. 488—489).

III. CONSTANCY AND FORMATION OF ABERRANT PLANTS.

1. FREQUENCY OF ABERRANTS.

Most of the progenies raised appeared to be quite uniform, making it easy to decide whether two such progenies growing side by side were



Figs. 1—4, cases of aberrant plants (to the right) and the corresponding normal plants (to the left). — Figs. 1—2, in a biotype from Kongsvold, Norway; Figs. 3—4, in a biotype from Mount Kerkau, Saltoluokta, Lapland.

morphologically identical or dissimilar. In several progenies, however, one or a few more or less deviating plants were sometimes observed, these aberrant plants usually being weaker than the normal sister plants (Figs. 1—4). Sometimes it was uncertain whether the aberrants were really genotypically deviating or mere modifications, but information on this point could be obtained by raising progenies from such suspected plants.

TABLE 3. *Frequency of aberrant plants.*

Region	Percentage of aberrants per progeny				Number of progenies	Total number of typical plants	Total number of aberrant plants	Percentage of aberrant plants
	0	0 — 10	— 20	— 30				
North Sweden	148	16	7	1	172	2670	35	1,29
Öland	32	10	1	1	44	696	18	2,52
Gotland	30	6	1		37	609	10	1,62

The frequency of aberrant plants was determined with the result given in Table 3. Out of 172 progenies of the North Swedish material (each progeny consisting of from 10 to 16 plants) 148 were quite uniform but in 24 progenies one or a few deviating plants did occur. The percentage of such plants per progeny was generally lower than 10. Out of 44 progenies from Öland 32 were uniform and 12 contained something between 0 and 30 per cent aberrant plants. The corresponding values from Gotland are 30 uniform progenies and 7 containing from 0 to 20 per cent aberrants.

Table 3 also gives the total number of typical and aberrant plants observed. These values also include the data from a few progenies containing less than 10 individuals. The percentages of clearly or possibly aberrant plants were found to be as low as 1,29 for North Sweden, 2,52 for Öland and 1,62 for Gotland. Thus, complete constancy is the rule, but now and then a few deviating plants will be formed.

2. CHROMOSOME NUMBERS AND VIGOUR OF THE ABERRANT PLANTS.

As the chromosome number was determined only in three plants per progeny, these plants being taken at random, it only happened in seven cases that root tips of the aberrant plants were fixed and the chromosome numbers determined. In five other cases progenies of the aberrant plants were raised and cytologically examined and allowed

conclusions as to the probable chromosome number of the mother plant. The following data were thus obtained:

Direct counts		Indirect conclusions	
Typical number	Aberrant number	Typical number	Aberrant number
38	35	38	36
40	38	33	31
37	35	44	42
41	37	33	49
41	42	33	± 50
33	33+1 f		
41	± 61		

The data demonstrate that in all these cases *the morphologically deviating plants were also cytologically deviating*, the aberrant morphology being accompanied and certainly caused by the changed chromosomal constitution. In one case the difference is very slight, an extra chromosome fragment being present in the aberrant (and its progeny; *cf.* below), in the other cases it is more obvious. This is especially true of the three cases in which the aberrant is ± *triploid* in relation to the typical plants, the three pairs of chromosome numbers being 41 — ± 61, 33 — 49 and 33 — ± 50. Otherwise the aberrant plants have usually somewhat *lower chromosome numbers* than the normal sister plants, this being observed in 7 cases out of 9. In one case the aberrant has one chromosome more than the normal sister plants, in the case already mentioned it had an extra fragment.

Finally, it should be mentioned that in two cases plants were found to have lower chromosome numbers than their sister plants although no morphological difference had been observed in the field. In one case the typical chromosome number was presumably 39, the aberrant number 37, the corresponding values in the other case being 37 and 35. In spite of these two cases, where a possible morphological difference may have been overlooked, it is obvious that a more or less slight change in chromosome number generally leads to a change in morphology.

In the first place the aberrant plants were detected on account of their *reduced vigour*, this reduction ranging from scarcely observable changes to quite poor dwarf plants. At the same time purely morphological changes were more or less obvious. Fertility was not studied in these plants, it may have been reduced but was at any rate sufficient to allow progenies to be raised without difficulty.

3. PROGENIES OF ABERRANT PLANTS.

Pairs of progenies from aberrant and normal sister plants were raised in 11 cases. In one of these the data are not yet conclusive. The remaining 10 cases may be grouped under the following headings.

A. CHROMOSOME NUMBERS OF ABERRANT PLANTS SOMEWHAT REDUCED. PROGENIES WITH POOR VIGOUR BUT PREDOMINANTLY CONSTANT.

Four cases belong to this category. *Case a* was observed in a strain from Ulriksfors (Jämtland) having $2n=38$. This chromosome number was found in three plants of the first generation and again in three other plants of a daughter progeny. This progeny consisted of 16 plants and was quite uniform. In the original progeny one plant had been denoted »aberrant»?, the chromosome number not being known. It gave rise to a progeny consisting of 14 plants of poor vigour, these plants being somewhat but not strongly variable. Chromosome counts were undertaken in 8 of these plants, 7 having $2n=36$ and 1 plant $2n=34$. Thus, the mother plant had been a true aberrant probably having $2n=36$ like most of its progeny.

Case b is represented by a strain from Kiruna (Lapland). Chromosome number of typical plants $2n=33$ (determined in 6 plants belonging to two generations). One plant in the first generation was denoted »probably aberrant, shorter and weaker than the sister plants». 18 daughter plants were raised which on an average had a very poor vigour and sharply differed from a sister plot represented by normal plants. 7 plants were cytologically examined and were all found to have $2n=31$. Thus, the observed loss of vigour was due to loss of 2 chromosomes.

Case c is represented by a strain from Dorotea (Lapland), the normal chromosome number of the strain being 37. Progenies were raised from one aberrant and one normal individual, the latter progeny consisting of 16 very vigorous and uniform plants. The aberrant mother plant gave rise to 7 daughter plants which were identical *inter se*, small and gracile and much weaker than the normal plants of this strain. All these plants were found to have $2n=32$ (or in one case ± 31).

Case d, finally, was met with in a strain from Nuolja (Lapland). The normal chromosome number of this strain is 44, this number being counted in 3 plants of the first generation and in 3 plants of the second generation. This latter progeny also comprised a plant with $2n=41$, which had died before the morphological observations were

made. One of the plants of the first generation was denoted »aberrant»? and gave a progeny of 20 plants, 2 of which died at an early stage, the remaining 18 plants on an average being very weak. 8 plants were fixed and found to have $2n=42$ (or in one case ± 42).

B. CHROMOSOME NUMBERS OF ABERRANTS SOMEWHAT REDUCED. PROGENIES POOR AND VARIABLE.

This category differs from the preceding one (A) by the occurrence of variation in the offspring of the aberrants. *Case a* is represented by a strain from Abisko (Lapland) in which the typical chromosome number is 38 (determined in 6 plants from two generations). The deviating plants had 35 chromosomes and gave rise to a weak and variable progeny consisting of 14 individuals. In 8 of these plants the chromosome number was determined, 2 plants having $2n=35$ like the mother but 6 plants being $\pm$ *triploid*, their chromosome numbers being ± 50, ± 51, ± 54, ± 55, 53, 53. Thus, the aberrant plant was semiapomictic, many unreduced ovules being fertilized.

Case b was met with in a strain from Nuolja (Lapland) having $2n=44$. A progeny was raised from a plant suspected of being aberrant, this progeny consisting of 20 plants. Vigour in this material was fairly good but variable and on an average less good than in the sister plot consisting of normal plants with 44 chromosomes. The $2n$ number was determined in 8 daughter plants of the aberrant and was found to range from 39 to 45, the following values being obtained: 39, ± 42, 42, 42, ± 45, ± 45, 45, 45.

C. ABERRANTS PROBABLY TRIPLOID GIVING MORE OR LESS CONSTANT PROGENIES.

In a strain from Nuolja (Lapland), representing *Case a* under this heading, the normal chromosome number was found to be 33 (5 plants of 2 different generations showing this number). One plant had been found to be aberrant (weak and with a deviating leaf shape) and from this individual 10 daughter plants were obtained which were slightly inferior in vigour compared with the normal plants in the sister plot. They also differed by having produced very few panicles. Chromosome counts in eight plants revealed that they were all triploid having 49 (or in 2 cases ± 49) chromosomes. This sharply indicates that the mother plant, the original aberrant, also was a triploid.

Case b, observed in a strain from Pårte—Aktse (Lapland) having

$2n=33$ (6 plants from two generations), originated from a plant suspected to be aberrant. A progeny of 18 plants was found to be constant and was not observed to differ from a sister plot with normal plants. Thus, the slightly deviating appearance of the supposed aberrant was first thought to be due to environmental influences only. However, the cytological control revealed that all the daughter plants were ± triploid, the following numbers being counted: 50, 50, 50, ±50, ±50, ±50, ±49, and ±47. The last-mentioned plant clearly deviated from 50 and evidently represented an aberrant in the otherwise rather uniform triploid offspring.

D. TWO OTHER CASES.

In two cases the aberrants had evidently only slightly deviating chromosome numbers but gave poor and variable progenies. *Case a* was met with in a strain from Frösön (Jämtland) in which the typical plants of the original progeny had $2n=33$ (determined in 6 individuals from two generations). The aberrant plants was examined twice with regard to the chromosome number, the first count giving $2n=\pm 33$, the second one $2n=33+1$ fragment. The offspring of this plant were poor and uneven but evidently in most cases, at least, represented the same chromosome constitution as the mother plant. In eight plants examined the chromosome counts gave the following result: $33+1$ fragment (4 plants), probably $33+1$ fragment (2 plants), $\pm 33+1$ fragment (1 plant) and ± 33 (1 plant). In the last-mentioned plant fixation was poor and the fragment may have been overlooked.

In *Case b*, finally, observed in a strain from Kvikkjokk (Lapland) the normal chromosome number was found to be $2n=40$ (observed in 5 plants of two generations). One plant suspected of being aberrant gave rise to a progeny of 12 plants, 5 of which died at an early stage. Of the remaining plants chromosome counts were available for four individuals having the $2n$ values 37, ±40, 41, and 42. Especially the plants with 37 and 41 chromosomes were morphologically clearly aberrant. Thus, the mother plant was certainly genotypically deviating but nothing definite can be said about its chromosome number.

IV. OCCURRENCE OF BIOTYPE GROUPS.

1. INTRODUCTION.

A morphological study of the material cultivated in the experimental field revealed an enormous morphological diversity which is

scarcely noticeable in the natural habitats. Owing to the apomictic mode of propagation, causing uniformity within plots, the differences between biotypes are generally very striking and clearcut. These differences are, of course, predominantly quantitative involving various dimensions of leaves, straws, panicles and spikelets. The biotypes also greatly differed in colour and colour distribution within the range green-yellow-red, the panicles could be quite erect or more or less decumbent, the panicle branches stiff or lax, *etc.* Also in general vigour there were all gradations from quite poor to exceedingly vigorous types.

On account of this great variability it was generally easy to decide whether two neighbouring plots were morphologically identical or not. In some cases, however, the differences were slight but by repeated observations in the same season and in two consecutive years it generally became clear whether such differences were significant or not.

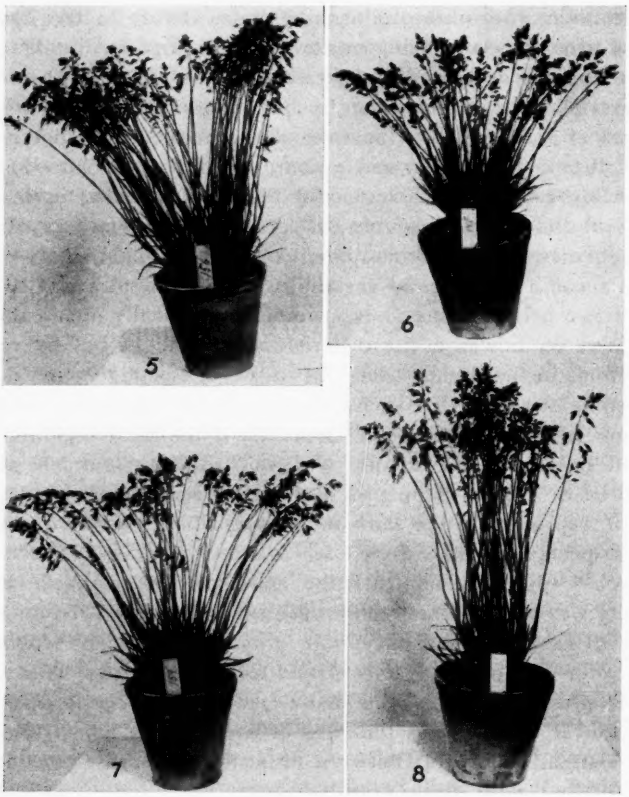
Especially among progenies from the same local region cases of complete morphological identity were not infrequent, and in addition to such cases it often happened that the progenies were only slightly different, representing the same main type which had been altered in minor respects in various directions. In a few cases, to be discussed in detail below (pp. 479—480), morphological identity or almost complete similarity was observed between progenies from widely different regions.

In all cases, without exception, *progenies considered to be morphologically identical were also found to have the same chromosome number*, whereas, on the other hand, *progenies differing in chromosome number were always found to be morphologically dissimilar.* In cases of slight differences between progenies, otherwise considered to represent the same main morphological type, there was generally identity between chromosome numbers. Deviations from this rule will be considered below.

As the constellations of morphological characters of the different progenies were strongly differentiated and very characteristic *it is highly probable that identity in morphology in this material really means genotypical identity.* This supposition is supported by the fact that such identity was most frequent between progenies of the same local region, in which the number of apomictic biotypes may sometimes be rather limited.

2. BIOTYPE GROUPS IN ÖLAND.

The Öland material observed comprised a total of 53 progenies which could be divided into 6 specific biotypes and 11 morphological



Figs. 5—8, biotypes with 41 chromosomes from Öland and Gotland. — Fig. 5 from Böda, Öland; Fig. 6, Persnäs, Öland; Fig. 7, a locality near Irevik, Gotland; Fig. 8, Sjonhem, Gotland. — Figs. 5 and 8 represent morphologically identical plants probably belonging to the same biotype. Fig. 7 shows a slightly deviating type, Fig. 6 is more strongly deviating but belongs to the main 41-chromosome type dominating in Öland and Gotland.

groups, comprising similar or identical biotypes. The majority of this material (35 progenies) belonged to the so-called 41-chromosome main type of Öland, the remaining 18 progenies forming some other groups or being specific. These categories will be dealt with separately.

A. THE 41-CHROMOSOME MAIN TYPE.

Although this category had certain characteristic features (evident to some extent from Figs. 5—6) it could be subdivided into 3 specific

biotypes and 7 groups comprising 32 progenies. Within these groups complete identity was only met with in regard of 8 progenies, similarity in regard of 24 progenies. Altogether the 35 progenies seemed to represent 30 different biotypes.

To be more precise, *Group I* comprised 7 biotypes (from Torslunda, Resmo and Stenåsa) which were all slightly dissimilar. *Group II* comprised 3 biotypes from Kastlösa, Högsrum and Räpplinge which were again similar but not identical. The same is true of 4 biotypes from Smedby and S. Möckleby constituting *Group III*. Three progenies from Kastlösa formed *Group IV*, and of these progenies two were quite identical, the third one slightly deviating. Groups V to VII are from the northern part of the island, *Group V* being composed of 5 progenies from Köping, Alböke, Föra and Persnäs (Fig. 6). Two of these, derived from two rather closely situated localities in Alböke, were identical and certainly represented the same biotype, the other three being slightly different *inter se* and also differing from the Alböke type. *Group VI* was composed of four progenies from Persnäs and one from Högby, all these evidently representing slightly different but closely related biotypes. *Group VII*, finally, was composed of five progenies from the Northwest part of Öland. Four of these [from Byxelkrok, North of Byxelkrok, Tokenäs and Böda (Fig. 5)] were found to be identical, whereas another progeny from Böda was found to be deviating.

Of the 3 specific biotypes belonging to the main Ölandic 41-chromosome type one, from Mörbylånga, may have had 40 instead of 41 chromosomes, but this deviation from the normal chromosome number could not be definitely established. The other two specific types were from Hulterstad.

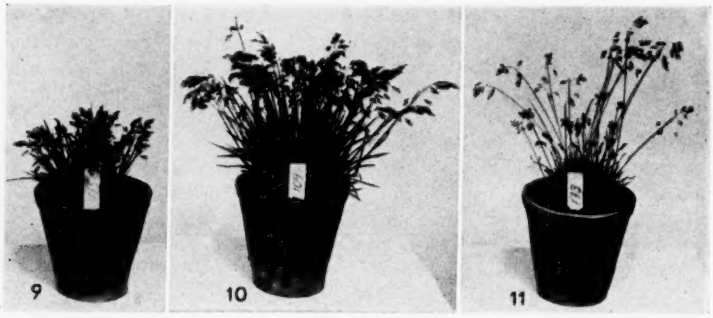
B. OTHER MORPHOLOGICAL GROUPS.

Several progenies did not belong to the main Ölandic type although their chromosome number was the same ($2n=41$). This was true of one group composed of more or less pronounced dwarf types (*cf.* Figs. 9—10) and of two other groups deviating in other ways. One group was highly interesting by having a clearly deviating chromosome number ($2n=35$) and also quite different properties. Finally, there were three specific biotypes, two of which had $2n=41$ but the third one $2n=43$.

Altogether the 18 progenies representing the present category B comprised 4 groups and 3 specific biotypes. There was complete identity in 6 cases, a more or less pronounced similarity in 11 cases. The total number of biotypes involved seemed to be 15.

The following details about Category B may be given. *Group VIII*, the dwarf type group, was composed of 7 progenies from Ventlinge (2 progenies), Vickleby, Mörbylånga, Hulterstad, Kastlösa and Högsrum. The progeny from Mörbylånga was identical with that from Hulterstad, the other progenies being slightly deviating in different ways. All these biotypes had $2n=41$.

Group IX had also 41 chromosomes and was composed of two identical pro-



Figs. 9—10, dwarf biotypes with $2n=41$ from Mörbylånga and Vickleby, Öland. — Fig. 11, a specific biotype with $2n=43$ from Broby, Fårö, Gotland.

genies from Högby and Byerum and one deviating biotype from Källa. *Group X* was from South Öland and comprised two slightly different biotypes from Ås and a third related biotype from Gräsgård, all three having $2n=41$. *Group XI* contained two evidently identical progenies from Stenåsa and Sandby having 35 chromosomes. This biotype does not only differ from all biotypes with 41 chromosomes by being much more gracile and delicate but also by having a different periodicity. All plants in these progenies (altogether 21 individuals) produced panicles already in 1945, in the same year as the seeds had been germinated, and did not survive the following winter. Almost all other plants of the Öland material did not reach full development until the summer of 1946 and only a few of them produced any panicles already in 1945.

Of the three independent lines from Alböke, Segerstad and Hulterstad the first two had $2n=41$, whereas the Hulterstad type had $2n=43$. This chromosome number was observed in the mother plant as well as in four of the daughter plants and was certainly characteristic of the entire progeny, consisting of 10 individuals. These plants were rather weak and several of them produced panicles already in 1945. Of the 10 plants, 7 survived the winter and were also alive in 1947, though still weak.

C. CONCLUSIONS CONCERNING THE ÖLAND MATERIAL.

In the first place the Öland material of *Poa alpina* is characterized by the occurrence of one dominating morphological type involving 35 of the 53 progenies observed. This type, although possible to describe, is not homogeneous but may be subdivided into several local groups. The members of these local groups are sometimes identical but more frequently slightly dissimilar. All these biotypes have $2n=41$, just as many of the remaining biotypes. These, however, were not considered to belong to the main Ölandic morphological type but formed other local groups or were represented by independent lines. One of these was

weak and had $2n=43$, instead of the usual number 41. The most interesting deviation, however, is represented by a weak and ephemeric biotype with 35 chromosomes, collected at two different localities in South Öland.

In spite of the group formation the genotypical polymorphism is evidently very marked in Öland, the 53 progenies representing not less than 45 different types. Cases of complete identity are met with occasionally but only with regard to neighbouring localities situated in the same or adjoining parishes.

In this connection it may be mentioned that, thanks to the courtesy of Dr. C. L. KIELLANDER, 17 living plants were obtained which had been collected along a road between Borgby and Alby, South Öland. These plants gave rise to 17 uniform progenies which were all morphologically identical and evidently represented the same biotype. This biotype was found to have 41 chromosomes, this number being obtained from 20 individuals representing nine of the progenies.

3. BIOTYPE GROUPS IN GOTLAND.

A. BIOTYPES WITH 41 CHROMOSOMES.

The material from Gotland shows essential similarities as well as dissimilarities in relation to the material from Öland. The first striking observation is the fact that *the 41-chromosome biotypes from Gotland belong to the same main morphological type as most of the Öland biotypes.* That this is not only a superficial morphological resemblance but rather the expression of a far-reaching genotypical similarity is quite obvious from the observations made and is indicated by the similarity between Figs. 5 and 8.

In the experimental field a progeny from Irevik, Gotland (No. 158) was situated rather close to two plots from Öland (No. 101 from Resmo and No. 156 from Böda). The similarity between this type from Gotland and the two biotypes from Öland was very striking, and between the Böda and Irevik progenies no morphological difference could be distinguished with certainty.

The great morphological similarity between 41-chromosome biotypes from the two islands is not limited to the progenies just discussed. It was soon realized that *the 41-chromosome progenies from Gotland were widely identical, most of them probably representing exactly the same biotype.* Of the 48 progenies from Gotland 27 represented this 41-chromosome type and of these 27 progenies from widely different

parts of the island not less than 20 were identical, the remaining 7 ones being deviating in various ways but nevertheless belonging to the same main type.

The 20 identical progenies were derived from the following localities: Irevik, Hangvar, Hall (2), Storugns, Ahr, Bunge (2), Fårö (2), Rute, Kyllej, Hejnum, Bro, Bro—Hejde, Hejdeby, Hejdeby—Visby, Österport (Visby), Torsburgen, Sjonhem (Fig. 8). (2) means that two progenies from different localities in the same parish were raised.

To this group seven slightly deviating progenies were attached, these progenies being derived from Snäckgärdet (Visby), Västerhejde, Vägome, Broa (Fårö), Fleringe, a locality 2 km. north of Fleringe and a locality 3 km. from Irevik.

It should be observed that the progeny from Bro—Hejde was found to have $2n=40$ instead of $2n=41$ as in all the other progenies. As far as the observations go, the lack of one chromosome in this type had no visible effect.

Another mystery is represented by a progeny from Fårö which, although derived from a single panicle, was found to be heterogeneous, comprising two different types. This may possibly be ascribed to an error at the planting, 3 plants representing another biotype.

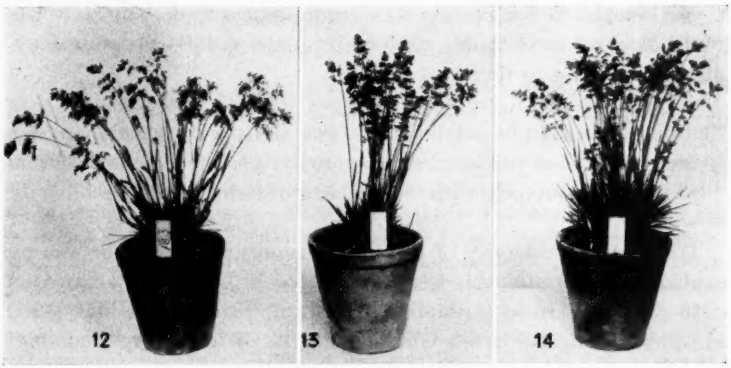
B. BIOTYPES WITH 33 CHROMOSOMES.

15 progenies, having $2n=33$, were all quite different from the 41-chromosome type but did not represent a uniform group. They could rather be subdivided into two well separated groups *a* and *b*, of which *a* resembled the 35-chromosome biotypes from Öland by being weak and slender and by producing panicles already in the first year. They did not die, however, in the following winter but persisted in all three years of observation.

Among the *a*-progenies formation of local groups was indicated, but on account of poor and uneven development the morphological comparisons were somewhat difficult. However, the following information may be given on this point.

Three progenies from Kräklingbo, Ardre and Ardre—Gammelgarn were clearly interrelated though somewhat different. A group of two slightly dissimilar biotypes from Vägome and Tingstäde—Hejnum is probably attached to the previous group through identity between the progenies from Ardre and Vägome. The Vägome type in its turn may be identical with two progenies from Ahr and Lickershamn, respectively. A progeny from Kappelshamn was similar to that from Lickershamn but slightly deviating. Another pair of related biotypes belonging to the same *a*-progenies was derived from Hejdeby and Hejnum—Fole (Fig. 12), respectively.

The *b*-group had only the chromosome number 33 in common with the *a*-group. They were rather vigorous, did not flower in the first season, had numerous, small, contracted and strongly anthocyanin-coloured panicles and blue-green leaves.



Figs. 12—13, biotypes with 33 chromosomes from Hejnum—Fole and Ardre, Gotland. These biotypes belong to different morphological groups. — Fig. 14, a biotype with with $2n=34$ from Storugns, Gotland.

Five different progenies belonged to this type, three being identical [from Ardre (Fig. 13), Katthammarsvik and Östergarn—Kräklingbo] and two deviating though identical *inter se*. The latter two were both from Etelhem.

Another 33-chromosome biotype (from Ardre) differed in several respects from the members of the *b*-group and was therefore considered to be specific.

C. SOME OTHER BIOTYPES.

An interesting pair of biotypes is represented by two progenies from Ljugarn and Ala, respectively. They were closely similar but not quite identical, the Ala type being stiffer and more erect. The chromosome numbers were $2n=44$ (Ala) and $2n=45$ (Ljugarn), two members not met with in any other biotypes from Gotland.

Two other specific chromosome numbers were represented by two biotypes from Storugns ($2n=34$, Fig. 14) and Broby—Fårö ($2n=43$, Fig. 11). The former one resembled the *b*-group among the 33-chromosome biotypes but was considered to be specific. The latter one was rather weak and like other slender types it flowered already in the first year. Otherwise, however, it was quite specific.

D. CONCLUSIONS CONCERNING THE GOTLAND MATERIAL.

Plants with $2n=41$ are dominating in Gotland as well as in Öland and it is obvious that the Gotland types with this number belong to the same main type as in Öland. One obvious difference between the Öland and Gotland types with this chromosome number is highly interesting,

the polymorphism within the 41-chromosome group being strong in Öland but weak in Gotland, almost all plants with 41 chromosomes in Gotland belonging to the same biotype.

A relatively large group of biotypes with 33 chromosomes occurs in Gotland and could be subdivided in two sharply different groups, one of which resembled the 35-chromosome biotypes from Öland. Finally, a few specific biotypes with other chromosome numbers have been met with.

Owing to the identity of most 41-chromosome progenies the polymorphism of *Poa alpina* is less pronounced in Gotland than in Öland, the 48 progenies from Gotland representing 23 different biotypes, the corresponding figures from Öland being 45 different types among 53 progenies.

4. BIOTYPE GROUPS IN NORTH SWEDEN.

The material from North Sweden was collected during a journey started at Östersund (Jämtland). Besides at Östersund (Frösön) seed material was gathered in the vicinity of railway stations between Östersund and Vilhelmina (South Lapland). From Vilhelmina an excursion was made to Saxnäs in the alpine region. Material was collected between Vilhelmina and Saxnäs, in the Saxnäs region and in the mountains North and South of Saxnäs (Marsfjäll and Borgafjäll).

After return to Vilhelmina the journey was continued with the inland railway to Jokkmokk, some material being collected again near the railway stations. More material was gathered in the Jokkmokk region, during a journey from Jokkmokk to Kvikkjokk, and especially during a walk of several days from Kvikkjokk to Saltoluokta. After collections in the Saltoluokta region the journey was continued to Abisko in Northern Lapland, where much material was collected during excursions in the region of Abisko, Björkliden and Låktajokko. After stays in Kiruna and Gällivare the journey was continued by bus to the Torne valley and from there by train to South Sweden. On the way back some material was obtained at Lännäs near Sollefteå (Ångermanland).

From the material collected during this journey a total of 108 progenies were raised from seeds and 17 progenies from bulbils of viviparous plants. Of the 108 agamospermic progenies 30 were apparently specific whereas 78 were members of a total of 20 morphological groups.

As before, the members of these groups were either identical with

other progenies or showed a more or less pronounced resemblance. The number of progenies per morphological group ranged from 2 to 20.

Group I was a large chiefly lowland or subalpine group mainly from North Jämtland and South Lapland comprising 20 progenies, of which the following were found to be identical:

Ulriksfors No. 2, Strömsnäs—Malgomaj, Malgomaj, Stalon (2 progenies), Saxnäs (2 progenies), Marsliden. All these progenies had $2n=38$ and the same was true of all progenies noted to show a more or less close resemblance to the above group. This »secondary» group consisted of 8 progenies from Ulriksfors No. 1, Stalon (2), Saxnäs, River Satsån, Satsfjäll, Marsliden and Kiruna No. 3. A »tertiary» group of 4 progenies was considered to resemble some of the members of the secondary group. Thus, a progeny from Jämtland's Sikås was similar to Ulriksfors No. 1 and a progeny from Marsfjäll was similar to the Satsfjäll progeny. In both these cases the chromosome number was again 38. The third member of the tertiary group was another progeny from Marsfjäll showing resemblance to the Marsliden progeny. In this case, however, there was a slight difference in chromosome number, the Marsfjäll progeny having $2n=39$. The fourth member of the tertiary group was a progeny from Bångnäs thought to resemble a progeny from Stalon. In this case, however, the morphological resemblance had been misleading, the Bångnäs progeny having $2n=33$.

Before leaving Group I, it should be observed that some members of the secondary group were found to be identical. This is true of River Satsån and Satsfjäll (two localities close to each other) and also of the pair Ulriksfors—Kiruna No. 3, Figs. 15—16 (two localities very widely separated).

Group II was less complicated than the previous one being composed of 4 progenies, all having $2n=33$. These progenies were represented by two pairs, one being composed of two identical progenies from Lännäs (Ångermanland), the other pair by progenies from Hoting (Jämtland) and Kiruna (No. 2), these progenies again being identical *inter se* (*cf.* Figs. 17—18) but slightly different from the previous pair.

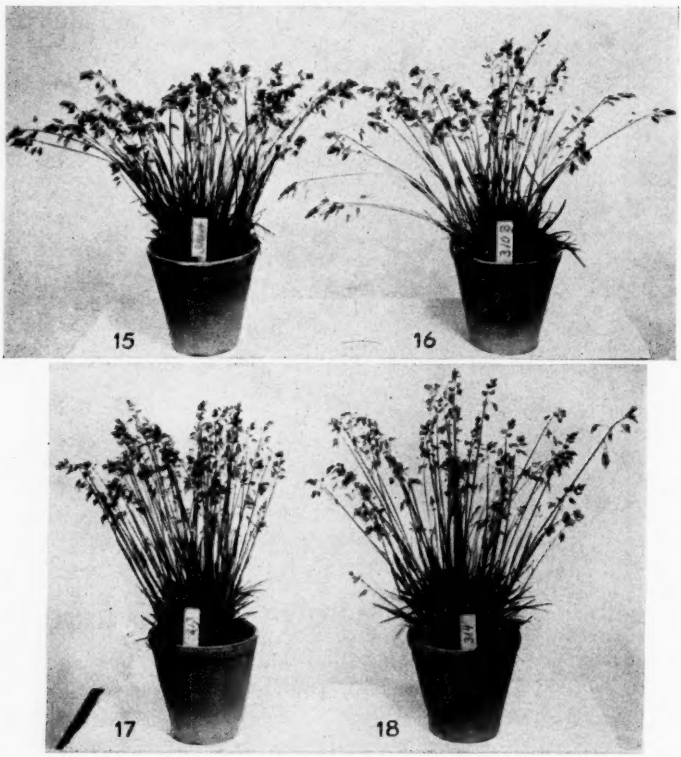
Group III comprised a primary group of 4 identical progenies with 38 chromosomes from Jokkmokk, Klubbudden and Kvikkjokk (2 progenies). With these progenies 6 other slightly deviating progenies were associated. 4 of these (from Jokkmokk, Björkholmen, Kvikkjokk—Pårte and Leipojärvi) had again $2n=38$ but the other two, from two localities at Kvikkjokk, had the slightly deviating chromosome number $2n=40$ and were identical *inter se*.

Two identical progenies from Harsprånget and Randijaur, having $2n=43$, belonged to *Group IV*, which also contained a secondary type with $2n=44$ from Abiskojokk and a tertiary progeny with $2n=45$ also from Abiskojokk. Thus, the 45-chromosome biotype was similar to the 44-chromosome one, which in its turn resembled the two progenies with $2n=43$.

Group V only contained two identical progenies with $2n=33$ from Jokkmokk and Njave, a place between Jokkmokk and Kvikkjokk.

Group VI comprised 4 progenies, all having 33 chromosomes. One progeny was from Aktse (between Kvikkjokk and Saltoluokta), the other three from the Abisko region. They were all slightly dissimilar.

Group VII was represented by 3 slightly dissimilar progenies from the Abisko region, all having $2n=38$.



Figs. 15—18, cases of identity between biotypes from widely separated localities. — Figs. 15—16, Kiruna No. 3, Lapland, and Ulriksfors No. 1, Jämtland; Figs. 17—18, Hoting, Jämtland, and Kiruna No. 2.

Group VIII was formed by two non-identical biotypes with 33 chromosomes from the Abisko region.

Group IX comprised a nucleus of 3 identical progenies with $2n=44$ from Mount Nuolja in the Abisko region. In this group was also included a fourth somewhat deviating progeny from Nuolja, also with $2n=44$, and a progeny from Torneträsk which was afterwards found to have 33 chromosomes.

Group X was composed of two identical progenies from Mount Nuolja, having $2n=37$, and a deviating progeny, also from Nuolja, which was afterwards found to have $2n=33$.

Group XI comprised one progeny from Kiruna and another somewhat similar progeny from an adjoining locality (Loussavaara) in the same town. Contrary to the rule, there was a difference in chromosome number, the former progeny having $2n=32$, the latter one $2n=38$.

Group XII contained two slightly dissimilar progenies from Gällivare, both having $2n=38$. *Group XIII* was composed in a similar way, comprising two similar progenies with $2n=33$ from Pajala and Tärendö, respectively. *Group XIV* was represented by two progenies from Mount Nuolja and Björkliden showing some morphological resemblance but differing in chromosome number ($2n=44$ and 38, respectively). *Group XV* comprised four progenies from Mount Nuolja, which were all found to be identical, representing a single biotype with $2n=39$. *Group XVI* contained two identical progenies with 33 chromosomes from the region of Pårte (between Kvikkjokk and Saltoluokta) and also a third progeny with the same chromosome number from the region between Pårte and Aktse.

Group XVII was much larger and comprised 11 progenies, all having $2n=38$, or in two cases possibly $2n=39$. It had a centre consisting of three identical progenies, 2 from Sitojaure and 1 from Saltoluokta and to this centre the other 9 progenies were related directly or indirectly. Of the 9 progenies 2 were from Saltoluokta, 4 from Låktajokko and 2 from Vassijaure.

Group XVIII contained 7 progenies of which the »central» ones were 5 identical progenies with $2n=44$ from Mount Kerkau and another locality in the Saltoluokta region. To this centre were attached two somewhat deviating progenies, one from Saltoluokta (again with $2n=44$) and another one from Abisko. The latter progeny was found to have a slightly deviating chromosome number, $2n=45$.

Group XIX was represented by two identical progenies with $2n=33$ from Övertorneå (Northernmost Sweden), *Group XX* by two similar progenies from Sorsele and Arvidsjaur, both having $2n=33$.

5. CASES OF IDENTITY BETWEEN PROGENIES FROM WIDELY SEPARATED LOCALITIES.

The members of the morphological groups were generally from the same local region. However, especially with regard to material from the lowland cases of morphological identity between progenies from widely different localities were also met with. Two striking cases of this kind are illustrated by Figs. 15—18. If the pictures had been colour plates instead of ordinary photos the similarity between the members of each pair would have been still more striking.

Fig. 15 shows a plant of a progeny from Kiruna, Lapland (»Kiruna No. 3») and Fig. 16 represents a progeny from Ulriksfors, Jämtland (»Ulriksfors No. 1»). The great similarity between these progenies was first observed in 1944 when Kiruna No. 3 was found to belong to the large morphological group I (*cf.* above p. 477). The morphological identity of the two types was observed before the identity of the chromosome numbers ($2n=38$) was known. In 1944 the two plots in question were situated rather far from each other in the experimental field but in spite of that the great morphological similarity was striking. This surprising fact was further tested by raising new progenies of the same types, these progenies now being planted side by side (in 1945).

The new plots were first studied in 1946 and were, indeed, found to be morphologically identical. The observations were repeated in 1947 on the same material and neither in this year could any morphological difference be detected. Without going into morphological details it may be mentioned that the biotype in question is characterized by a low and procumbent mode of growth and by lax and heavy panicles with a small amount of anthocyanin.

The other case, illustrated by Figs. 17—18, is represented by progenies from Hoting (Jämtland) and another type from Kiruna (No. 2). This biotype ($2n=33$) is also rather low but the straws are stiffly erect, the leaves bright green and strongly folded and the panicles vividly red-coloured. As in the previous case the great morphological similarity was first observed in 1944, and by studies of new progenies in 1946 and 1947 the conclusion was reached that we have here again a case of one single biotype growing at localities as far apart as approximately 480 kilometers. The distance between Ulriksfors and Kiruna is of the same order af magnitude.

It should be observed that a biotype from Lännäs, Ångermanland, is also *very* similar to the Hoting—Kiruna 2-pair. Only by repeated morphological studies of progenies representing two generations it became possible to discern some slight and probably significant differences, the Lännäs type having a still stronger panicle colour and probably being somewhat shorter than the material from Hoting and Kiruna. Nevertheless, the Lännäs type must be genotypically closely related to the Hoting—Kiruna type. Thus, the members of the morphological group II were derived from three localities situated very far apart. As already mentioned, the distance between Hoting and Kiruna is about 480 kilometers, the corresponding values for Hoting—Lännäs and Kiruna—Lännäs being about 125 and 545 kilometers, respectively. It should be observed that certain lowland types of *Poa alpina* may have become widespread by railway transports, several of the biotypes just discussed being collected near railway stations (those from Hoting, Ulriksfors and Kiruna).

Within the other morphological groups cases of absolute identity always represented progenies derived from the same local region. Some cases of rather close similarity were found, however, between progenies derived from areas as far apart as the Saltoluokta and Abisko regions. In one case of this kind (Group XVIII) the morphological difference was correlated with a change in chromosome number from 44 in the Saltoluokta region to 45 in the Abisko region.

V. DEGREE OF POLYMORPHISM IN DIFFERENT GROUPS OF MATERIAL.

The degree of polymorphism in the *Poa alpina* populations of Öland and Gotland has already been discussed and it was, for instance, pointed out that there is a much stronger polymorphism among the 41-chromosome biotypes of Öland than among those of Gotland. With regard to the material from North Sweden the occurrence of the same biotype at widely separated lowland localities seemed to indicate that the frequency of different biotypes in the lowland might be rather limited. On the other hand, a strong polymorphism was evident when surveying progenies from alpine localities (*cf.* Figs. 19—25). An attempt to verify or disprove this apparent contrast was made by comparing the ratio of specific and group-forming biotypes of different regions. In doing this, the material was roughly divided into the following four categories: *A*, biotypes from the lowland, *B*, biotypes from subalpine localities, *C*, biotypes from the alpine region in South and Middle Lapland (around Saxnäs, Kvikkjokk and Saltoluokta), and *D*, biotypes from the alpine region in North Lapland (around Abisko).

A. BIOTYPES FROM THE LOWLAND.

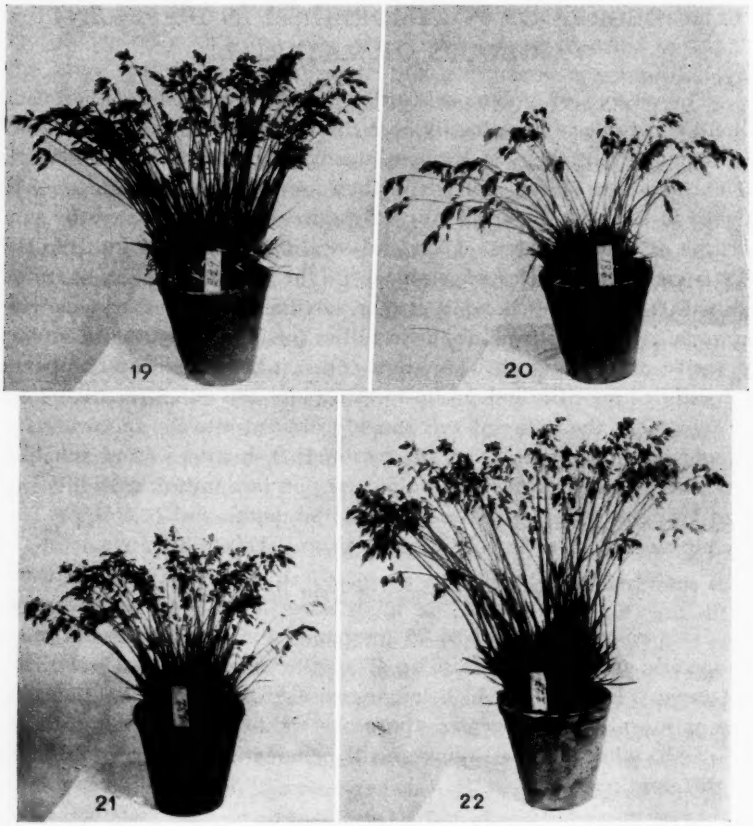
This category comprised 22 progenies, 6 of which were found to be specific and 16 group-forming. The latter ones belonged to 6 different groups. Eight of them, belonging to 4 groups, were identical with one or more other progenies, the other eight were only more or less similar to other biotypes. Consequently, the entire material represented 18 different types.

As the distinction between lowland and subalpine localities is not sharp it may be useful to enumerate the localities representing category *A*: Frösön, Jämtland's Sikås, Hoting, Ulriksfors (2 progenies), Dorotea, Strömsnäsbruk, Sorsele, Arvidsjaur, Kiruna (4 progenies), Luossavaara, Gällivare (2 progenies), Pajala, Tärendö, Leipojärvi, Övertorneå, Lännäs (2 progenies).

B. BIOTYPES FROM SUBALPINE LOCALITIES.

This material was represented by 23 progenies, 1 of which was specific, the other 22 group-forming. These progenies belonged to 5 different groups and 15 of them (belonging to 5 groups) were identical with other progenies, 7 progenies only showed a certain degree of similarity. Consequently, this category comprised 13 different types.

The 23 progenies were derived from plants growing at the following localities: Lake Malgomaj (at the School of Agriculture), Stalon (4 progenies), Bångnäs, Njaka-



Figs. 19—22, four different biotypes from Marsfjäll, South Lapland, having 49, 44, 38 and 38 chromosomes, respectively.

fjäll (near Bångnäs), Saxnäs (3 progenies), Marsliden, a locality near Marsliden, Jokkmokk (3 progenies), Harsprånget, Randijaur, Klubbudden, Björkholmen, Njave, Kvikkjokk (2 progenies), Nammatj (near Kvikkjokk).

C. BIOTYPES FROM THE ALPINE REGION IN SOUTH AND MIDDLE LAPLAND.

This region, ranging from Borgafjäll in the South to Saltoluokta in the North, was represented by 31 progenies, 16 of which were specific and 15 group-forming, involving 6 different groups. Of these 15 progenies 8 (belonging to 4 groups) showed identity with one or

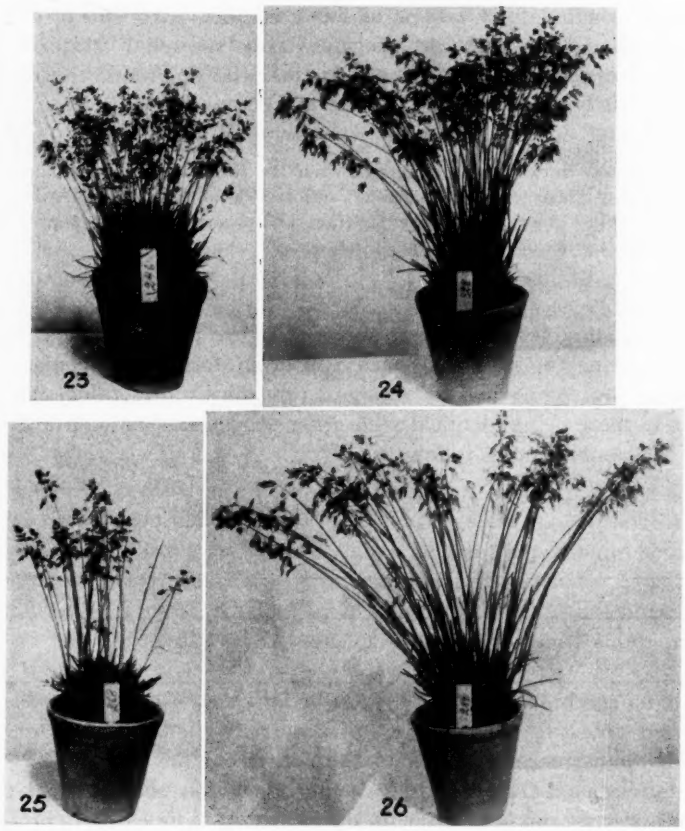


Fig. 23, a biotype from Pårte, Lapland ($2n=38$); Fig. 24, Jokkmokk No. 2, Lapland, ($2n=33$); Fig. 25, a Nuolja biotype, Lapland, having $2n=33$; Fig. 26, a biotype from Dalfors, Dalecarlia, $2n=44$.

more other progenies, 7 only a certain degree of similarity. Thus, the whole material comprised 27 different types.

The material was gathered at the following localities: River Satsån, Satsfjäll, Marsfjäll (6 progenies), Borgafjäll (2 progenies), the region Kvikkjokk—Pårte (6 progenies), the region Pårte—Aktse (3 progenies), Sitojaure (3 progenies), the Saltoluokta region (9 progenies).

D. BIOTYPES FROM THE ALPINE REGION IN NORTH LAPLAND.

This category comprised 32 progenies, 7 of which were specific and 25 group-forming, involving 5 groups. Of the latter ones 7 pro-

genies, belonging to 3 groups, showed identity with one or several other progenies, whereas 18 progenies only showed a more or less pronounced similarity. Thus, the entire material was found to comprise 28 different types.

The localities involved in the present category are more closely situated than in the previous categories, as is evident from the following enumeration: Abisko (2 progenies), Abiskojokk (4 progenies), different localities near Abisko (6 progenies), Nuolja (12 progenies from different altitudes), Björkliden (3 progenies), Låktajokko (4 progenies), Pallemvagge (1 progeny).

The data given above were analysed in two different ways, firstly, by comparing the ratios of specific to group-forming progenies, and, secondly, by comparing the ratios of progenies representing different types to those being identical with other progenies. The data available may be summarized as follows:

Region	Specific	Group-forming	Non-identical	Identical
A	6	16	18	4
B	1	22	13	10
A+*B*	7	38	31	14
C	16	15	27	4
D	7	25	28	4
C+*D*	23	40	55	8

It seems reasonable to add the data from the two really alpine categories *C* and *D* and to contrast the values thus obtained with those from lower regions (*A*+*B*). This shows that *specific biotypes are more numerous, and hence the polymorphism is more pronounced, in the alpine region than at subalpine localities and in the lowland.* The difference between the ratios 7 : 38 and 23 : 40 is significant, a χ^2 test giving the χ^2 value 5,75 which corresponds to a P intermediate between 0,02 and 0,01.

The same final result is obtained from a comparison between the frequencies of non-identical : identical progenies, the ratios to be compared in this case being 31 : 14 and 55 : 8. A χ^2 test in this case gave a χ^2 of 5,49 and a P value again intermediate between 0,02 and 0,01.

It should be observed that the categories *A*, *B*, *C*, *D* are rather unequal with regard to the geographical range covered by them, and that this inequality will tend to reduce the actual difference in poly-

morphism between the alpine region and lower regions. Thus, category *A* is represented by lowland progenies from widely different parts of North Sweden (situated in the provinces of Jämtland, Lapland, Norrbotten, and Ångermanland). Category *B* is much less extensive and mainly represents two subalpine areas, one from South Lapland (the region between Vilhelmina and Saxnäs) and one from Middle Lapland (the region around Jokkmokk and between Jokkmokk and Kvikkjokk). Under such circumstances it is not surprising that category *A* seems to be somewhat more polymorphic than *B*.

There is a similar difference between the two alpine categories *C* and *D* of which *C* represents a much wider range of localities than *D*, which is rather strictly localized to the region around Abisko. It is probable that this difference in magnitude of geographical range is responsible for the difference in frequency of specific biotypes. A χ^2 test of the ratios concerned, 16 : 15 and 7 : 25, gave a χ^2 of 6,01 and a P intermediate between 0,02 and 0,01.

There is no difference between categories *C* and *D* in regard of the ratios between non-identical and identical progenies. This probably implies that within the more narrow Abisko-region the progenies showing differences are often sufficiently interrelated to be included in various morphological groups, whereas in the more heterogeneous category *C* the average differentiation is stronger and specific biotypes therefore more frequent.

The most appropriate comparison between material from an alpine environment and material from a lower altitude is probably between categories *B* and *C*, which in the main represent the same geographical area in South and Middle Lapland. The ratios of specific : group-forming progenies in these categories are 1 : 22 and 16 : 15. A χ^2 test in this case will result in a χ^2 of 13,68 and a P smaller than 0,001, the difference thus being quite significant. A similar χ^2 test of the ratios 13 : 10 and 27 : 4 representing frequencies of non-identical and identical progenies showed this difference to be slightly less significant than the previous one, χ^2 being 6,43 and P intermediate between 0,02 and 0,01.

Thus, although a much larger material would have been desirable and the morphological classification in several cases must be regarded as tentative rather than quite exact, the present data certainly strongly indicate that *the highest concentration of different biotypes of Poa alpina is to be found in the alpine region.*

VI. CHROMOSOME NUMBERS IN DIFFERENT GROUPS OF MATERIAL FROM NORTH SWEDEN.

1. SEED-FORMING BIOTYPES.

In the preceding paragraph differences in frequency of biotypes in various regions was demonstrated. It is of interest to examine whether these differences are also associated with differences in chromosome number. As already stated (p. 461), there is a wide variation in chromosome number in this material, the $2n$ values ranging from 32 to 50. The distribution of these numbers in the different regions, *A*, *B*, *C*, *D*, was found to be the following (Table 4).

TABLE 4. *Chromosome numbers in mother plants from different regions in North Sweden.*

Region	Chromosome number: 32	33	34	35	36	37	38	39	40	41	42	43	44	45	...	48	49	50	n	M
A (lowland)	1	7	—	—	—	4	10												22	35,95
B (subalpine)		3	—	1	—	1	14	—	2	—	—	2							23	37,78
A + *B*	1	10	—	1	—	5	24	—	2	—	—	2							45	36,89
C (alpine)		8	—	—	—	—	11	3	—	—	1	—	6	—	—	1	—	1	31	38,74
D (»)		8	—	—	—	2	7	5	—	—	—	—	6	4					32	38,84
C + *D*		16	—	—	—	2	18	8	—	—	1	—	12	4	—	1	—	1	63	38,79

The chromosome numbers given are not from individual plants as in Table 1 but represent the numbers of the mother plants. In some cases (when living plants were collected) chromosome counts were undertaken in the mother plant itself as well as in three daughter plants, in other cases (when seed material was collected) the chromosome number of the mother plant was concluded from the chromosome numbers in the offspring, three daughter plants being examined.

Table 4 shows that in the lowland (category *A*) the chromosome numbers were found to range from 32 to 38 with an average $2n$ value of 35,95. In the subalpine region, *B*, the chromosomal variation is similar but a few numbers higher than 38 occur, and the average, 37,78, is somewhat higher than in the lowland. In the alpine region *C* numbers higher than 38 are still more frequent, and the average number, 38,74, has surpassed 38. In the alpine region *D* the situation is about the same, the average chromosome number being 38,84.

It remains to be tested whether the differences observed are really significant. As the series in question certainly do not show a normal distribution, allowing calculations of m-values, but rather two or three maxima (at 33 and 38 in *A* and *B*, and in *C* and *D* a third maximum at 44) a χ^2 test must instead be undertaken. It seems natural then to group the material as follows:

Category	Chromosome numbers 32—35	37—40	42—50	n
A	8	14		22
B	4	17	2	23
A+*B*	12	31	2	45
C	8	14	9	31
D	8	14	10	32
C+*D*	16	28	19	63

As before, *A* and *B* may be added to one series which is contrasted with the sum of *C* and *D*. This will give a χ^2 value of 11,81 which for 2 degrees of freedom corresponds to a P value intermediate between 0,01 and 0,001. This result clearly demonstrates that on an average the chromosome numbers in the alpine region are higher than in material from lower levels.

Now, this calculation was based on the actual representation of the chromosome numbers in the different regions without paying attention to the fact that several progenies evidently represented the same biotype. If, instead, we examine the chromosomal variation of different biotypes occurring in the various regions the following result is obtained (Table 5).

TABLE 5. *Chromosome numbers in biotypes from different regions in North Sweden.*

Region	Chromosome number: 32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	n	M
A (lowland)	1	5	—	—	—	4	8													18	36,06
B (subalpine)		2	—	1	—	1	7	—	1	—	—	1								13	37,46
A + *B*	1	7	—	1	—	5	15	—	1	—	—	1								31	36,85
C (alpine)		7	—	—	—	—	9	3	—	—	1	—	5	—	—	—	1	—	1	27	38,89
D (»)		8	—	—	—	1	7	4	—	—	—	—	4	4						28	38,54
C + *D*		15	—	—	—	1	16	7	—	—	1	—	9	4	—	—	1	—	1	55	38,71

A comparison between Tables 4 and 5 shows that the average chromosome numbers of the different regions are approximately the same. To make a χ^2 test possible the values of Table 5 may be condensed to the following distributions:

Category	Chromosome numbers 32—35	37—40	42—50	n
A	6	12		18
B	3	9	1	13
A+*B*	9	21	1	31
C	7	12	8	27
D	8	12	8	28
C+*D*	15	24	16	55

A χ^2 test of the distributions 9 : 21 : 1 and 15 : 24 : 16 gave a χ^2 of 8,93 which corresponds to a P intermediate between 0,02 and 0,01.

Thus, with a high degree of probability it may be concluded that *on an average the alpine biotypes have a higher chromosome number than biotypes from lower regions.*

This difference involving biotypes is only slightly less significant than the difference involving progenies.

2. VIVIPAROUS BIOTYPES.

In Table 2 (p. 462) the chromosome numbers determined in a total of 67 viviparous plants from North Sweden were given, these numbers ranging from 38 to 56 with apparent maxima at 39, 46—47 and 56. These 67 plants belonged to 17 progenies and some of these progenies were morphologically identical, probably representing the same biotype.

The 17 progenies were derived from the following localities and were found to have the following chromosome numbers:

Pårte ($2n=46$), Aktse ($2n=45$), Saltoluokta ($2n=46$), Mount Kerkau near Saltoluokta ($2n=56$), Abisko ($2n=47$), Mount Nuolja near Abisko (5 progenies having $2n=38$, ± 41, 46—47, 54 and 56, respectively), Pallemvagge ($2n=56$), Pallemjokk ($2n=\pm 41$), Björkliden ($2n=56$), Låktajokko (2 progenies, both having $2n=46$—47), Vassijaure (2 progenies having 39 and ± 46 chromosomes, respectively). One of the progenies from Vassijaure, two progenies from Nuolja and the one from Abisko were in such a poor condition in the experimental field that morphological comparisons were excluded. The remaining 13 progenies were found to represent 9 different biotypes, some of the progenies being identical. This is true of the Pårte and Saltoluokta progenies, both having $2n=46$. The Aktse progeny with $2n=\pm 45$ was rather similar but anyhow clearly different.

Another group of probably identical progenies, all having 56 chromosomes, was represented by the material from Björkliden, Pallemvagge and one of the Nuolja progenies. Finally, the two Låktajokko progenies were morphologically identical, both having $2n=46$—47.

With regard to chromosome number the data just given may be summarized in the following two series:

	Chromosome numbers												
	38	39	40	41	...	45	46	46-47	47	...	54	55	56	*n*
All progenies:	1	1	—	2	...	1	2	4	1	...	1	—	4	17
Different biotypes (among 13 of the progenies):		1	—	2	...	1	1	2	—	...	—	—	2	9

As the chromosome numbers, on an average, are higher than those obtained in the seed-forming material it remains to examine the significance of this difference. For a comparison of the two categories it is convenient to divide the material in four groups corresponding to the maxima observed. This will result in the following series

	Chromosome numbers				
	32—35	37—40	41—50	54—56	*n*
Seed-forming progenies	28	59	21		108
Viviparous		2	10	5	17

To allow a statistical treatment these series must be condensed to the distributions 87 : 21 and 2 : 15 representing $2n$ values below and above 41. This gives a χ^2 of 33,9 which corresponds to a P smaller than 0,001. Thus, it is certain that the viviparous progenies examined actually had higher chromosome numbers than the seed-forming ones.

It would have been still more desirable to test whether such a difference is true not only of progenies but also of biotypes. However, as only 9 viviparous biotypes were available for such a comparison the material is too small for a statistical treatment. Nevertheless, the data obtained indicate that *an increased number of chromosomes is characteristic of the viviparous types of Poa alpina growing in North Sweden.*

VII. SOME DATA CONCERNING MATERIAL FROM NORWAY, ICELAND AND GREENLAND.

Although rather fragmentary some information may be given concerning material from Norway, Iceland and Greenland. The *Norwegian material* was mainly represented by 8 progenies representing grand-

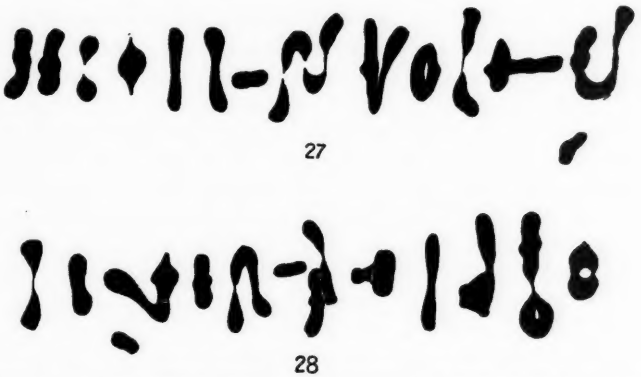
daughters of plants from Kongsvold collected by Dr. C. L. KIELLANDER. Although the original mother plants had been growing within a rather limited area the eight progenies were found to represent six different biotypes, and three or four different chromosome numbers. To be more precise, there was one morphological group consisting of three identical progenies having $2n=33$. A second group consisted of two similar but not identical progenies, one with 33 and the other with ± 32 chromosomes. Three other progenies were specific and were found to have the chromosome numbers 33, 37 and 39, respectively.

Another progeny from Jotunheimen was specific and had the chromosome number ± 43 (counted in five plants representing two generations). S. Knutshø is a third Norwegian locality represented in my material by a single progeny having $2n=33$.

Thus, as might be expected, the Norwegian types of *Poa alpina* show a similar chromosomal variation as the Swedish material. Local groups of similar or identical progenies were also met with and the progenies have the usual apomictic uniformity. Occasional deviating plants also occurred. One such $\pm$triploid aberrant with 52 chromosomes is included in Table 1, showing the chromosome numbers of all 36 Norwegian plants examined.

The material from *Iceland* had been collected by Dr. A. LÖVE at nine different localities far from each other in Middle and North Iceland. At each locality several living plants were taken and sent together in a bunch in which it was not possible to separate the original individuals. Hence, root-tip fixations from the nine collective samples in several cases gave rise to different chromosome numbers for each sample. Thus, in »Iceland 6», for instance, different roots had 33, 35, 38 and 39 chromosomes. This demonstrates the occurrence of a chromosomal polymorphism at this locality and similar results were also obtained for others of the nine collective samples. Hence, in order to analyse the material a total of 34 progenies were raised from single panicles of the original collective samples. These progenies showed the apomictic uniformity, were partly identical with each other but also represented a number of different morphological types.

This material has not been sufficiently studied to warrant a detailed discussion. At present we must confine ourselves to the statement that the chromosome numbers showed a similar variation as in Sweden and Norway. Seven different numbers were met with ranging from 33 to 44. (Table 1.) Several of the original collective samples contained viviparous individuals but as these viviparous plants turned out to be grow-



27

28

Figs. 27—28, first metaphase complements (separately drawn) in a biotype from Hoting, Jämtland, having $2n=33$. — Fig. 27, $3_{IV}+1_{III}+8_{II}+2_{I}$; Fig. 28, $3_{IV}+3_{III}+5_{II}+2_{I}$.

ing together with seed-forming individuals in the original samples their chromosome numbers are not known with certainty.

Unfortunately very little can be said about the small samples of *Poa alpina* from *Greenland*. Of 4 living plants, kindly put to my disposal by Drs. JÖRGENSEN and WESTERGAARD, 3 produced a few panicles and gave rise to three apomictic and uniform progenies. Two of these were probably identical having 42 or 43 chromosomes. The third progeny was different and had a higher chromosome number (± 46). These chromosome numbers are within the range found in North Sweden but rather on the plus side.

VIII. MEIOTIC OBSERVATIONS.

From previous work (MÜNTZING, 1940, pp. 123—125) it is known that meiosis in the apomicts of *Poa alpina* is irregular and that multivalents are frequent, indicating a partially autopolyploid constitution. In a biotype from Pajala with $2n=33$ the average number of multivalents (quadrivalents and trivalents) was found to be 2,6 and the average number of univalents as high as 3,7. These univalents divide at I-A. Similar conditions were met with in another biotype from Korpilombolo ($2n=38$) and in a biotype from Mösseberg having $2n=33$. In the latter one multivalents containing more than 4 chromosomes were also observed.

To these scattered observations made previously it may now be

added that in another biotype with $2n=33$ from Hoting (Jämtland) meiosis followed a similar course as in the Pajala biotype. Two complete I-M configurations are represented in Figs. 27—28. In the first one there are probably $3_{IV}+1_{III}+8_{II}+2_{I}$, in the other one $3_{IV}+3_{III}+5_{II}+2_{I}$. In a third metaphase group the configuration $1_{IV}+3_{III}+8_{II}+4_{I}$ could be distinguished. In 11 cells at I-A the number of lagging univalents with tendency to divide ranged from 1 to 6 with an average of 2,64.

IX. POLLEN FERTILITY.

In 1944 pollen fertility was studied in 84 progenies of *Poa alpina* from North Sweden, the chromosome numbers of these progenies ranging from 32 to 45. In most cases pollen samples were taken from 3 plants per progeny. Data from aberrant plants are not included in the table, such aberrants generally having less good pollen than typical plants. Besides determining the percentage of apparently good pollen grains, it was also noted for a number of plants that the good pollen grains were obviously variable in size, and in a few of them this variation was found to be very strong.

TABLE 6. *Chromosome number, pollen fertility and pollen variability.*

Chromosome number	Per cent good pollen: 20	30	40	50	60	70	80	90	100	n	M	Variable size: Abs.	Variable size: Per cent	Strongly variable: Abs.	Strongly variable: Per cent
32—35	1	—	—	3	1	3	15	42		65	88,38	56	86,2	8	14,3
37—40	1	2	1	—	—	3	6	112		125	92,12	88	70,4	11	12,8
42—45								33		33	95,00	12	36,4	—	0,0

The data gathered in this way are summarized in Table 6. As before, the material has been divided into three chromosome classes corresponding to the three maxima occurring in this material. A positive correlation between chromosome number and pollen fertility is indicated, the lowest chromosome class having 88,38 per cent good pollen, the intermediate class 92,12 per cent and the highest class 95,00 per cent. The significance of this correlation was tested with the χ^2 method, using the classes 20—90 and 90—100 per cent good pollen. In this way the material will be represented by the following distributions:

Chromosome number	Per cent good pollen 20 — 90	— 100
32—35	23	42
37—40	13	112
42—45	0	33

The χ^2 for heterogeneity will in this case be 27,17 which for 2 degrees of freedom corresponds to a P smaller than 0,001. Thus, it is certain that *in the material studied there is a positive correlation between chromosome number and pollen fertility.*

Pollen sterility is not only more pronounced in the biotypes representing the lowest chromosome numbers but the apparently good pollen grains are also more variable in size than in biotypes with higher chromosome numbers. Thus, in the biotypes with 32—35 chromosomes 86,2 per cent of the pollen samples were classified as containing pollen grains of variable size. In the next chromosome class with $2n=37$—40 the corresponding percentage is 70,4 and in the biotypes with the highest chromosome numbers it is as low as 36,4. A χ^2 test of this heterogeneity will be based on the following distributions:

Chromosome number	Non-variable	Variable
32—35	9	56
37—40	37	88
42—45	21	12

This gives a χ^2 of 25,87 which corresponds to a P smaller than 0,001. Thus, it is certain that in the present material *plants with low chromosome numbers, on an average, had a more variable pollen size than those with higher chromosome numbers.*

Table 6 also contains data concerning plants with »strongly variable» pollen size. This category is most frequent in the 32—35 class and is not represented at all in the 42—45 class. The values are too small for statistical treatment but evidently support the previous conclusive evidence that plants with relatively low chromosome numbers have a strong tendency to form pollen of variable size.

X. CHROMOSOME NUMBER AND MORPHOLOGY.

In 1944 detailed measurements were undertaken in the 84 progenies of the North Swedish material. The following characters were examined: length of straw, panicle length, length of the basal panicle branch, length of spikelet, length, breadth and index of the uppermost

leaf, thickness of 10 leaves, thickness of culm. These characters were measured in 10 plants per progeny and the average value used in attempts to correlate chromosome number and various morphological characters. These attempts, however, were almost completely unsuccessful, and in most cases there was no significant correlation. Such was the case with regard to chromosome number on one hand and on the other hand thickness of leaves and straw, spikelet length, panicle length and leaf index. In one single case a significant positive correlation was established. This was between chromosome number and straw length, the latter character probably being positively, though weakly, correlated with general vigour. The coefficient of correlation in this case was found to be $+0{,}36$ and a test of the significance of this correlation coefficient gave a P intermediate between $0{,}01$ and $0{,}001$. It should be observed that the correlation studied was between chromosome number and straw length in different biotypes, not different progenies. Thus, the average values of different but identical progenies were combined to a general average representing the biotype in question.

The positive correlation between chromosome number and straw length was also tested with an analysis of variance, the material being condensed to the following three series (Table 7).

TABLE 7. *Correlation between chromosome number and straw length among biotypes from North Sweden.*

Chromosome number	Straw length in mm. 275	300	325	350	375	400	425	450	475	n	M
32—35	2	2	3	2	4	3	2			18	366,7
37—40		2	1	2	4	7	9	5		30	412,5
43—45			1	1	2	1	3			8	(400,00)
Total	2	4	5	5	10	11	14	5		56	

Working with a provisional average value of 387,5 the mean square for variation between series will be 19,03 and for within series 3,03. This gives a v^2 value of 6,28 which again corresponds to a P intermediate between $0{,}01$ and $0{,}001$. It is obvious that this heterogeneity is mainly represented by the marked difference between the 32—35 series and the 37—40 series. This in its turn mainly goes back to a difference between 16 biotypes with $2n=33$ and 21 biotypes with $2n=38$.

Whether the correlation observed is a direct effect of the difference in chromosome number or due to special genes which happen to be

more frequent among the biotypes with higher chromosome numbers is still an open question. The latter alternative, however, seems to be the most plausible one as no effects of the differences in chromosome number could be traced on such characters as thickness of leaf and culm and leaf index which are otherwise generally influenced by quantitative alterations in chromosome number.

XI. DISCUSSION.

1. *Degree of apomixis in various biotypes of Poa alpina.* — Not only the viviparous biotypes but also the seminiferous ones are apomictic, but in the seed-forming biotypes apomixis is not absolute, a certain proportion of deviating plants, »aberrants», being formed. In a previous experiment (MÜNTZING, 1940, pp. 116—119) four of eight strains studied seemed to be perfectly constant in chromosome number, this constancy being accompanied by a complete morphological uniformity. In four other strains, however, a certain proportion of aberrants with slightly deviating morphology were produced, the frequency of aberrants being different in different strains. Thus, in a strain from St. Gothard, Switzerland, the percentage of aberrants was as high as $25{,}93 \pm 8{,}43$, whereas in a strain from Mösseberg, Sweden, it was as low as $4{,}35 \pm 3{,}01$. The difference is rather significant, P being intermediate between 0,01 and 0,02.

The present data, obtained from numerous biotypes from North Sweden, Öland and Gotland, demonstrate that apomixis in this material is quite predominating, the percentages of clearly or possibly aberrant plants being as low as 1,29 for North Sweden, 2,52 for Öland and 1,62 for Gotland. Thus, complete constancy is the rule but now and then a few deviating plants are formed.

In material from Switzerland the degree of apomixis is rather variable and, on an average, less pronounced than in the Scandinavian material. As reported earlier (MÜNTZING, 1940, pp. 138—145), some strains were quite or almost constant, while others showed a more or less marked degree of aberrant formation. The strain from St. Gothard has already been mentioned. In another strain from Arosa 22 individuals were cytologically examined, and of these only 12 had the typical number 26 or ± 27, the remaining ones (representing 45 per cent of the plants) being deviating in chromosome number as well as morphology. Still earlier (MÜNTZING, 1932) a strain had been obtained from Fürstenalp, Switzerland, which was found to be quite sexual, the chromosome

number oscillating between 22 and 31. However, it was evident from crosses between two plants of this strain and a Swedish apomict (MÜNTZING, 1940, pp. 145—155) that a certain proportion of unreduced ovules are formed by the sexual strain which, therefore, is not quite free from apomictic tendencies.

There exist, however, purely sexual strains in the *alpina* group, one of them being diploid with 14 chromosomes in the root tips and with some additional accessory chromosomes in the »germ line» (MÜNTZING, 1946, 1948, 1949). This strain is probably native in the mountain regions of South Russia. Without difficulty it could be crossed with a North Swedish apomict as the male parent.

It is highly interesting that another diploid strain of *Poa alpina* was recently detected in Switzerland by Dr. A. NYGREN (unpublished). If this strain, which according to NYGREN belongs to the variety *xerophila* of *Poa alpina*, should turn out to be sexual, which is probable, this may help to explain why, on an average, the Swiss representatives of *Poa alpina* are less strongly apomictic than the Scandinavian biotypes. Crosses between this or other similar strains and Swiss apomicts may lead to segregation products with variable degrees of sexuality. At any rate there seem to be rather continuous transitions between complete sexuality and complete apomixis in *Poa alpina* just as in *Poa pratensis* (ÅKERBERG, 1941).

2. *Nature of aberrants and their mode of reproduction.* — As a rule, the aberrants are the result of occasional fertilizations of egg cells, in rare cases they are represented by haploids originating from the parthenogenetic development of reduced but unfertilized egg cells. In cases of fertilization the gametes which unite may both be reduced, the resulting plants generally deviating slightly from the typical chromosome number of the strain. Theoretically, the chromosome number in such cases may be unchanged but on account of meiotic irregularities, which seem to be of general occurrence in *Poa alpina* (*cf.* below, p. 510) it is much more likely that the aberrants will have somewhat higher or lower numbers than the mother strain. Owing to meiotic elimination of univalents, lower numbers should be more frequent than higher ones. In cases of fertilization the gametes which unite may also be unreduced or represent a combination of one unreduced and one reduced gamete. In the latter case it is generally the ovule which is unreduced.

In the material studied previously (MÜNTZING, 1940) the nature and frequency of the aberrants were investigated in a collection of strains from Scandinavia and Switzerland. On account of unsatis-

factory fixation apparent deviations with one, or in a few cases with two chromosomes from the typical number were not reliable. Stronger deviations, however, represented by 33 plants, were significant and were found to be distributed in the following way:

± diploid		± triploid		± tetraploid		
—	+	—	+	—	0	+
8	3	9	2	7	4	0

This means that of the 33 plants 11 were ± diploid, 11 ± triploid and 11 ± tetraploid. The number of ± diploid aberrants may in reality have been somewhat higher owing to the difficulties with the chromosome counts. The differentiation into — and + subcategories indicates if the aberrants in question had lower or higher numbers than the exactly diploid, triploid or tetraploid chromosome number. In the tetraploid category some plants had exactly the tetraploid number (indicated by a 0), otherwise the total ratio between — and + is 24 : 5. Most probably, and as expected, this significant difference is caused by meiotic elimination of chromosomes. The exact tetraploids, 3 of which were observed in the same progeny, may perhaps be the result of somatic doubling rather than union of unreduced gametes. However, unreduced pollen grains have been observed and even found to function in a certain plant with $2n=24$ (MÜNTZING, 1940, pp. 137—138).

In the present material the chromosome numbers were known by direct counts or indirect conclusions in a total of 12 aberrants. Three of them were triploid (2 on the — side, 1 on the + side), the remaining ones were ± diploid, 7 being on the — side, 2 on the + side. Thus, we have here again a preponderance of minus aberrants owing to meiotic elimination and again diploid as well as polyploid aberrants. However, for unknown reasons the latter category was less frequent now than in the previous material.

In *Poa pratensis* all categories of aberrants have been found to be more or less completely sexual, the progenies of such aberrants generally being highly variable in chromosome number as well as morphology (ÅKERBERG, 1939, 1941; MÜNTZING, 1940). Preliminary evidence from progenies of aberrant individuals in the Swiss material of *Poa alpina* indicated a similar behaviour (MÜNTZING, 1940, pp. 140—144) but was not quite conclusive. More information on this point was obtained from the present material.

As described in detail above (pp. 466—468), 10 pairs of progenies from aberrant plants and normal sister plants could be divided into

different categories. Much to our surprise the aberrants were more apomictic than expected, though to different degrees in different cases. The first category was denoted »Chromosome numbers of aberrant plants somewhat reduced. Progenies with poor vigour but predominantly constant». Four of the cases belonged to this category and two to the next category, »Chromosome numbers of aberrants somewhat reduced. Progenies poor and variable». In one of these cases the variable offspring of the aberrant comprised a majority of ± triploid plants, in the other case the offspring were only represented by ± diploid individuals. An interesting case is represented by an aberrant differing cytologically from the normal plants only by the presence of an extra fragment. All the daughter plants seemed to have the same chromosomal constitution. Another category, finally, is represented by probably triploid aberrants, giving more or less constant progenies. Thus, surveying the whole material it is obvious that *in aberrants of Scandinavian apomicts of Poa alpina the apomictic tendencies are, on an average, stronger than the sexual ones.*

3. *Chromosome numbers in the Swedish material.* — Before the present paper only the chromosome numbers 33, 38 and 35 were known from Sweden, and of these the number 33 seemed to be especially interesting as it had been found to occur in material from widely different parts of the country (North Sweden, Gotland and Västergötland; MÜNTZING, 1932, 1940). The present results verify that 33 and especially 38 are frequent among the seminiferous biotypes, but several new numbers were also found. Thus, in North Sweden besides the maxima at 33 and 38 there is a third maximum at 44, and also the number 39 is rather frequent. Many other numbers have been observed but in a low frequency.

The number 35, previously obtained in a biotype from Öland, was found again in new material from this island. However, another chromosome number, 41, is quite dominating in Öland, and the same number prevails in the island of Gotland. The number 33 is also rather frequent in Gotland and was again met with in some different biotypes from Västergötland, another province in South Sweden. In Öland and Gotland a few other chromosome numbers occur but only exceptionally.

Thus, it is evident that the chromosome numbers of the Swedish *alpina* biotypes are not distributed at random but that one or a few numbers may be dominating in various regions. Under such circumstances it is rather clear that identity in chromosome number between populations of different regions indicates a definite relationship. This

is most obvious with regard to the 41-chromosome biotypes, occurring in Öland as well as Gotland, but also the biotype groups with 33 chromosomes, occurring in widely different parts of the country, may have a common origin.

In the viviparous biotypes the chromosome numbers were on an average definitely higher, ranging from 38 to 56. Among the seed-forming biotypes 50 was the highest number met with. Some chromosome numbers in viviparous *Poa alpina* from Sweden have already been published by NYGREN (1950, p. 7). Seven clones were examined by him and found to have chromosome numbers ranging from 42 to 54—57. One of these clones (from Vassijaure, $2n=46$) may be identical with one of my clones, the others are from localities not represented in my material. Thus, the data obtained by NYGREN strengthen the conclusion that the spectrum of chromosome numbers occurring in the viviparous biotypes is significantly different from that occurring in the seminiferous biotypes.

4. *Chromosome numbers in material from other countries.* — The material from *Norway*, *Iceland* and *Greenland*, studied by myself, was rather restricted but seemed to represent approximately the same range of chromosome numbers as in North Sweden, $2n$ values from 33 to 46 being observed. BÖCHER (1938) found $2n=33$ in a non-viviparous form from Angmagssalik in East Greenland. BÖCHER and LARSEN (1950), however, report $2n=28$ for another non-viviparous *Poa alpina* from West Greenland, growing at 67° North near the margin of the inland ice. This is a very interesting finding which certainly deserves further study. BÖCHER (1938) also reports the chromosome number ± 48 in a viviparous type from the *Faroe Islands*. FLOVIK (1938) studied two viviparous clones from *Spitzbergen*, having $2n=44$ and $42+4$ fragments, respectively. He also reports the number 33 for another viviparous type from arctic Norway. In his list of chromosome numbers in viviparous *alpina*, NYGREN (1950) includes 5 clones from Norway. Four of them, from Mt. Knutshø (Dovre), had from 36 to 38 chromosomes, a fifth type from Northern Norway (Mt. Javreoaivve) had a higher number ($2n=52$).

A total of 12 progenies (10 seminiferous and 2 viviparous) from Switzerland were studied by MÜNTZING (1932, 1940). Two viviparous clones had $2n=26$ and 33, respectively. Seven of the seed-forming progenies were characterized by the number 37, one progeny by 33 and one by 26. One strain was sexual with chromosome numbers oscillating between 22 and 31. To this should now be added *Poa alpina* var. *xero-*

phila which according to NYGREN (unpublished) is a diploid with 14 chromosomes. Thus, in Switzerland the average chromosome number in the *Poa alpina* populations is most probably lower than in Scandinavia, though many of the strains more or less constantly have 37 chromosomes, a number not known to prevail in any other region.

Highly interesting results concerning the *Poa alpina* population in the Tatra and Pieniny mountains in Poland have recently been obtained by SKALINSKA (1949—50, 1952) who investigated 111 viviparous and 2 seed-forming types from this rather restricted mountain region, which is quite isolated from other areas in which *Poa alpina* is growing. The chromosome numbers of the two seed-forming types were 22 and 33, respectively. The viviparous types showed the following distribution (SKALINSKA, 1952, p. 256):

$2n$:	14	22	26	28	33	34	35
Number of clones:	1	35	32	16	24	5	2

In the first place we notice the occurrence of one diploid clone with 14 chromosomes, the first example of vivipary in a diploid grass (*cf.* GUSTAFSSON, 1947, p. 71). Secondly, the chromosome numbers are conspicuously low in relation to the corresponding values from Scandinavia, the highest number in the Polish material being lower than the lowest number found among viviparous biotypes from Sweden and Norway. The Polish values are low even if they are compared with the seed-forming biotypes from Scandinavia and also seem to be lower than in most strains from Switzerland, in which the number 37 was found to be predominating.

SKALINSKA believes that the biotypes with 28 chromosomes are autotetraploids of the single biotype with $2n=14$. This is quite possible, and perhaps the viviparous clones with 56 chromosomes occurring in Sweden represent products of another doubling of the chromosomes.

In order to explain the numbers 22 and 33, SKALINSKA (1952) refers to the finding by MÜNTZING (1940, pp. 136 and 182) of a case of secondary polyploidy in a sexual strain from Switzerland. In this strain with oscillating chromosome number a cytologically stable strain with $2n=22$ could be selected. Meiosis in this strain was regular, 11 bivalents being present at first metaphase. SKALINSKA suggests that the viviparous biotypes with 22 chromosomes represent such secondary diploids, and if this is true the biotypes with $2n=33$ might be secondary triploids. This view gains some support not only by the frequent occurrence of the number 33 in Scandinavia and elsewhere (Switzerland, Greenland

and Iceland) but still more from the fact that in North Sweden one of the three chromosome number maxima was situated at 44. In conformity with SKALINSKA's hypothesis such plants would represent secondary tetraploids. However, more data are needed before this hypothesis can be considered to be plausible. It is also doubtful whether the Polish biotypes with $2n=26$ are really derived from hybrids between the types with 22 and 33 chromosomes, a hypothesis also suggested by SKALINSKA.

5. *Chromosome number and ecology.* — SKALINSKA's investigations of *Poa alpina* in the Polish mountains are not restricted to mere chromosome counts but mainly deal with the ecological properties of biotypes with different chromosome numbers. Thus, the diploid biotype was quite rare and occurred in a sheltered valley at a height of about 900 m. At the same locality biotypes with 22, 26 and 28 chromosomes were present. The 22-chromosome type in general manifests a well marked preference for habitats in relatively lower situations and is considered to represent a subalpine ecotype. Types with 26 chromosomes, on an average, reach a greater height but are highly variable in this respect. Thus, for instance, at Western Tatra they go further down than at High Tatra, where they grow at alpine localities. Types with 28 chromosomes are almost exclusively found on lime-stone and at medium height. Types with 33—35 chromosomes, finally, represent a single ecological group, which is distinctly alpine. Further, it shows a preference for acid soils on granite at High Tatra. Of the two seed-forming biotypes the one with 22 chromosomes was found on lime-stone at West Tatra and the one with 33 chromosomes on granite at High Tatra.

This thorough investigation leaves no doubt that viviparous biotypes with different chromosome numbers have different ecological properties, and it also demonstrates that the biotypes with the highest chromosome numbers reach higher altitudes than types with lower numbers.

This result has a parallel in the seminiferous *Poa alpina* plants of North Sweden. As was demonstrated above (pp. 486—488), material from the alpine region has, on an average, higher chromosome numbers than material from subalpine localities or the lowland. The difference is rather slight, it is true, and the same low numbers occurring at lower altitudes also occur in the *regio alpina*. However, in addition, higher numbers are present at alpine localities which were not found at lower levels and, hence, the difference in average

chromosome number between alpine and lower regions was found to be significant.

In *Poa pratensis* there also seems to be a difference between the chromosome numbers in material from the alpine region and the lowland. According to NYGREN (1950), high chromosome numbers are more frequent in subspecies *alpigena* than in *eu-pratensis*.

The viviparous biotypes of *Poa alpina* in North Sweden are characterized by still higher chromosome numbers and are generally growing at especially humid localities in the alpine region. According to NANNFELDT (1940), however, *Poa alpina* in Norway goes down to the coast, where humidity is also great. Hence, the high numbers may represent an adaptation to extreme humidity rather than to high altitude. On the other hand the great difference in chromosome number between the viviparous types in Poland and Scandinavia probably implies another example of a positive correlation between latitude and degree of polyploidy.

The extensive literature concerned with this problem has been reviewed by LÖVE and LÖVE (1949). These authors also point out that in corresponding plant communities the frequency of polyploids seems to increase under extreme edaphic conditions. Thus, for instance, polyploids, on an average, seem to be better adapted to hydrophytic conditions than diploids. This may explain part of the average preponderance of polyploids in arctic regions, in which the localities are often very humid. GUSTAFSSON (1948) also finds that hydrophytes are made up of polyploids to a very high extent. This may perhaps be a direct consequence of the lowering of the osmotic pressure which is characteristic of autoployploids (*cf.* MÜNTZING, 1936, pp. 295—296). This view is somewhat supported by the ecological properties of intraspecific chromosome races in *Galium palustre*. As first demonstrated by FAGERLIND (1934, 1937), this species is represented by several different chromosome races, *e. g.* diploids with 24 chromosomes and octoploids with 96. FAGERLIND observed that the octoploids grow out in the water among the *Phragmites* vegetation, whereas the diploids prefer relatively dry habitats on the shore. Quite analogous results were obtained by HANCOCK (1942) with regard to diploid, tetraploid and octoploid races growing in England.

A nice contrast to the high-polyploid viviparous *Poa alpina* types adapted to humid conditions is represented by the new diploid strain found by NYGREN in Switzerland. Judging from the variety name *xerophila*, it must be adapted to dry habitats.

The apparent correlation between polyploidy and humidity preference in *Poa alpina*, however, is much disturbed by the fact that the dominating type in Öland and Gotland has a relatively high chromosome number (41) in spite of the fact that this material is growing at localities which during most of the summer are quite dry.

It should not be forgotten, however, that in most cases the superiority of the polyploids is not in the first place due to the polyploidy as such but to the genetic constitution of the various genomes present in the polyploid strain (*cf.* MELCHERS, 1946; LÖVE and LÖVE, 1949). Already from the classical investigation of BABCOCK and STEBBINS (1938) on *Crepis* we know that the superiority of polyploid apomicts in this genus is primarily due to the fact that the polyploids combine the ecological properties of different diploid species. CLAUSEN (1952), working with species crosses in *Poa*, has given new examples of the same phenomenon. Thus, for instance, the F_1 hybrid *Poa ampla*×*pratensis* was found to have an excellent ecological ability surpassing that of the parent species. The hybrids of this combination are largely produced by the union of unreduced eggs of *ampla* with reduced pollen of *pratensis* and they have, therefore, from 90 to 100 somatic chromosomes as compared with 63 and 68 in its two respective parents. Many F_1's of this combination are apomictic and have a remarkable tolerance to differences in climate, provided the climates are fairly continental. In climates with very severe winters they outgrow the parents, and at lower altitudes their performance is also very creditable.

This recalls the investigation by SOKOLOVSKAJA and STRELKOVA (1938) who found a very high frequency of polyploids in the high mountain regions of Pamir and Altai. The authors consider that polyploids will more readily than diploids adapt themselves to unfavourable climatic, temperature and other conditions of life. One of their examples is from the genus *Poa*, in which *Poa sibirica* with $2n=28$ ranges mostly over the meadow-lands of valley territories, whereas *Poa alpina*, *altaica*, *tibetica* and others were found to have $2n=42$ and to be native at high mountain meadows.

Not only in *Poa alpina* but also in other species there is a tendency for viviparous species or biotypes to have higher chromosome numbers than their seminiferous relatives. Such is the case in *Festuca ovina* (TURESSON, 1930, 1931) where the seed-producing forms were found to be diploid, the viviparous triploid, tetraploid and hexaploid with a gradual increase in the degree of vivipary with increasing chromosome number. FLOVIK (1938) has also observed a viviparous *ovina*

form with $2n=49$. In the viviparous *Deschampsia alpina* NYGREN (1949 a) found the chromosome number to range from 39 to 56, whereas the non-viviparous species of the genus have lower chromosome numbers. NYGREN believes that the chromosome races of *D. alpina* are to be looked upon as polyploid derivatives of *D. caespitosa* having $2n=26$. As in *Festuca*, the degree of vivipary was found to increase with increasing chromosome numbers.

As emphasized by NANNFELDT (1940), the rule that viviparous types have higher chromosome numbers than related non-viviparous types is not without exceptions, and with regard to the biotypes of *Poa alpina* occurring in Poland and Switzerland no difference in chromosome number between the two categories could be found. However, comparisons in these cases are premature. In Switzerland the viviparous category was only represented by 2 biotypes and in Poland it was the other way round, the viviparous biotypes being in great majority and only 2 seminiferous biotypes so far being investigated.

6. *Degree of polymorphism in different areas. Biotype groups.* — In the preceding paragraph it was pointed out that in *Poa alpina* the same range of chromosome numbers was found in the alpine region as at lower altitudes but also higher numbers not occurring at lower levels. Already from this fact it is rather evident that the degree of genotypical polymorphism must be higher in the alpine region than in the lowland. This was definitely proven by means of morphological studies, which showed that groups of identical or similar biotypes were frequently met with in material from the lowland or subalpine localities, whereas in the alpine region such groups were fewer and more restricted to narrow local areas. The same fact may also be expressed by saying that the number of morphologically specific types were more frequent at high altitudes than at low ones. Thus, it is evident that in the lowland the frequency of different biotypes is relatively restricted, and this explains why in certain cases progenies from widely different regions were found to represent the same biotype (pp. 479—480).

This fits in rather nicely with the view expressed by TURESSON (1927) that the glaciations meant greater destructions to the lowland ecotypes, while the alpine ones were able to survive in ice-free refuges. A number of species populations were entirely deprived of their lowland ecotypes and are, therefore, now only represented by arctic and alpine ecotypes. The lowland ecotypes of other species populations were not entirely lost but became poor in biotypes. It seems rather likely that *Poa alpina* may belong to this category. Another possibility might

be that new biotypes have arisen in the alpine population which had such a constitution that they could establish themselves in the lowland. However, in view of the presumably very severe selection during periods of glaciation it is rather unlikely that genes favouring lowland life were still present in the alpine populations.

Another interesting difference in the degree of polymorphism between different areas was found with regard to the 41-chromosome biotypes occurring in the islands of Öland and Gotland. Whereas the Öland material of this kind could be subdivided into several different morphological groups the corresponding material from Gotland was much more uniform, most progenies belonging to one morphological group which was identical with some of the types from Öland. This must mean, either that once a larger number of biotypes came to Öland than to Gotland or, more probably, that the 41-chromosome biotypes in Öland are older than those in Gotland, and that Gotland has secondarily received its 41-chromosome biotypes from Öland. In this probable emigration from Öland only one or a few biotypes may have been represented and this has resulted in a rather homogeneous population in Gotland which has not yet become much differentiated.

It should be observed, however, that there is also another quite different category of *Poa alpina* in Gotland, represented by biotypes having $2n=33$. This material, which has no counterpart in Öland, is split up into two main morphological types. No special morphological similarity can be found between these types and material from Västergötland or North Sweden having the same chromosome number. Yet the peculiar aneuploid chromosome number indicates that far back all these types may be derived from related ancestors. One tentative possibility is that they are derived from various secondary diploids with $2n=22$, in which the production of types with 33 chromosomes might easily occur through fertilization of unreduced ovules. Some »triploid» aberrants in the present material were found to be quite or almost quite apomictic and true-breeding (p. 467) and, hence, 33-chromosome types arisen in the way suggested might be constant immediately.

As the concentrations of different biotypes is lower in some regions than in others the question may be raised whether a *taxonomical treatment* of this material would be possible. In a few cases something of this kind might be done. Thus, in Gotland the 41-chromosome type might probably be taxonomically distinguished from the two main groups of biotypes with 33 chromosomes and these are rather different

inter se. The 41-chromosome type in Öland has already been described by TURESSON (1927) as »*Poa alpina* oect. *pediaca*», and the slender, almost annual 35-chromosome type from Öland might perhaps also be distinguished as a special variety. A few of the biotype groups occurring in the lowland in North Sweden might perhaps be taxonomically distinguished but would probably be difficult to recognize in the field, most characteristica being of a quantitative nature. In the alpine region, finally, the polymorphism is certainly so great that a taxonomical treatment of this polymorphism is quite excluded.

In another apomictic *Poa* species, *Poa arctica*, conditions are somewhat similar to those in *alpina* and here NANNFELDT (1940) has been able to distinguish several subspecies, six of which represent the Scandinavian biotypes. Three of these are glacial survivors from Central Norway which only represent remnants of a pre- or inter-glacial *Poa arctica* population once more richly differentiated. One of these subspecies is now quite uniform, is viviparous and has only one chromosome number (NYGREN, 1950). The other two subspecies are seed-forming and totally apomictic, each comprising biotypes with a narrow range of different chromosome numbers. Other *arctica* populations, outside Scandinavia, have been found to be too complex for taxonomical treatment. NANNFELDT (1940) ascribes this to the occurrence of partial sexuality and intercrossing, which results in a great polymorphism which cannot be taxonomically entangled.

7. *Formation of new biotypes*. — The most obvious source of new biotypes in *Poa alpina* as well as in other more or less apomictic species of *Poa* is probably the occasional occurrence of fertilization of reduced or unreduced egg cells leading to the appearance of morphologically and cytologically deviating aberrants. In *Poa pratensis* such aberrants were always found to be sexual but in later generations some descendants may revert to apomictic propagation again (observations by CLAUSEN and collaborators, cited from NYGREN, 1949 b, p. 297). As demonstrated above, the situation in *Poa alpina* is somewhat different, many of the aberrants, and even »triploids», being totally or predominantly apomictic. In such cases new biotypes may arise in one step. The trouble is only that in practically all cases observed by me such aberrants and their progeny were clearly inferior in vigour and would not have a chance to survive under natural conditions.

However, as an enormous number of deviating aberrants are produced every year in the natural populations of the species, it is quite likely that a few of them will survive and will enrich the species popu-

lation. Indications of this are represented, *e.g.*, by some apomicts with deviating chromosome numbers observed in Öland and Gotland. One plant collected at Hulterstad, Öland, had 43 chromosomes, instead of 41, and produced a constant but rather weak progeny which probably would have been eliminated rather rapidly in its original habitat. From Gotland some biotypes were also found to be deviating in a similar way. A biotype from Bro—Hejde had 40, instead of 41, chromosomes, but as far as the observations go the lack of one chromosome had no visible effect in this case. A 34-chromosome biotype from Storugns was similar to certain biotypes with 33 chromosomes but was considered to be specific and had evidently fairly good vigour. Another biotype from Broby—Fårö had $2n=43$ and was rather weak and slender. All these cases probably go back to aberrants which had appeared under natural conditions.

The fact that the chromosome numbers 41 and 33 are nevertheless quite predominant in Gotland suggests that such aberrants and their progenies are in most, perhaps all, cases more or less ephemerical, and that the differentiation of new successful biotypes may instead predominantly occur by mutation. It must be admitted, however, that this view is only supported by indirect evidence. No new mutant types with changed morphology, unchanged chromosome number and good vigour have been observed to arise in my cultures. However, they must be more difficult to distinguish than the clearly deviating aberrants which in most cases are conspicuous by their reduced vigour. It is also difficult to imagine how the marked polymorphism within the main 41-chromosome type in Öland could have arisen by fertilization and genetic recombination without change of chromosome number. It seems more plausible to assume that the polymorphism has slowly and gradually been increased by mutations affecting a small fraction of the enormous seed material formed each year. The same process may also be responsible for much of the polymorphism in North Sweden and elsewhere. However, the great range of different chromosome numbers in these regions also indicates that fertilization and recombination may be at work.

It is also possible that somatic non-disjunction and elimination of single chromosomes may be important. This was stressed by SKALINSKA (1952) who mentions that in several cases the chromosome number in the root tips showed a certain degree of variation within the individuals.

Such processes, fertilization or somatic changes in chromosome number, may explain certain cases in which members of the same mor-

phological group are slightly dissimilar and at the same time show slight differences in chromosome number. Such was the case in Group III of the North Swedish material with the chromosome numbers 38 and 40 (*cf.* p. 477). The same phenomenon was repeated in Group IV (p. 477) in which slightly different biotypes were found to represent the numbers 43, 44 and 45.

As the range of habitats is more varied in the large alpine and subalpine regions it is not improbable that fertilization and recombination will have a better chance to produce viable and valuable products in such areas than in such restricted and relatively uniform regions as Öland and Gotland in which mutation rather than recombination may lead to a differentiation of the material.

In the related species *P. pratensis* the formation of more or less sexual aberrants is certainly of great importance for the preservation of the polymorphism. Evidence has also been obtained that some derived sexuals have good vigour and fertility (ÅKERBERG, 1941) and it is further realized that sexual individuals may produce year after year a very large number of different recombinations some of which may be successful. Apomixis and sexuality are in this species not antagonistic but coordinate phenomena (GUSTAFSSON, 1947). The same is probably true also of *Poa alpina*, though in this species the cooperation between apomixis and sexuality may be more successful in some regions than in others.

8. *Chromosome number and morphology.* — TISCHLER (1954) includes *Poa alpina* among the so-called »dysploid» plant species which are characterized by unusual variability in chromosome number but which do not show any clear morphological or physiological effects of this chromosomal variation. Such is, indeed, the situation in a sexual strain from Switzerland which had an oscillating chromosome number and in which no correlation between chromosome number, vigour and fertility could be found (MÜNTZING, 1940, pp. 128—130). In the present material measurements of a series of morphological characters were undertaken in 84 progenies from North Sweden. As this material represented a range of different chromosome numbers attempts were made to find correlations between chromosome number and the various characters measured. Only in a single case a significant coefficient of correlation was obtained, plants with higher chromosome numbers having a longer straw than those with lower numbers. It was suspected, however, that this result was caused by special genes rather than by the chromosome number as such. This suspicion is strengthened by

results obtained by SKALINSKA (1952) who in her material found that the straws were shorter in biotypes with high chromosome numbers than in those with low numbers. Otherwise SKALINSKA found it impossible to draw conclusions concerning the probable chromosome number of a biotype from its morphology. However, biotypes with high chromosome numbers were characterized not only by shorter straws but by having thicker and stiffer straws, longer panicles and coarser bulbils. Especially the thicker straws and the coarser bulbils indicate a direct effect of the higher chromosome number, causing the cells to be larger.

It might be imagined that such effects are more likely to appear in a viviparous material in which the original effects of an increased chromosome number are not levelled out by genetic recombination occurring to a certain extent among seminiferous biotypes.

The absence or low degree of correlation between chromosome number and morphology in a variable material found in nature does not imply that the chromosomes in question are genetically inert. The following circumstances demonstrate that they are still active:

In sexual strains containing plants with a range of different chromosome numbers the morphological variation is much greater than in apomictic strains only representing a single chromosome number (*cf.* MÜNTZING, 1932, pp. 137—138). The apomictic strains are, indeed, morphologically exceedingly uniform but sometimes contain single morphologically deviating individuals. In all cases studied these aberrants were found to have deviating chromosome numbers and thus the generally slight change in chromosomal constitution must be responsible for the morphological alteration. It must be admitted, however, that in a few cases there was evidence of a change in chromosome number which had escaped observation. Such cases were exceptional and chiefly concerned $\pm$triploid individuals which seem to be more difficult to distinguish than aneuploid aberrants.

The morphological comparison between different progenies and the possibility of uniting various progenies to morphological groups also has a bearing on the present problem. Thus, it was found that progenies which were morphologically identical always had the same chromosome number. This would not have been expected if the chromosomes had been inert. Secondly, if two progenies differed in chromosome number they were always morphologically dissimilar. Several cases were met with in which progenies were morphologically similar and obviously related but nevertheless showed a difference sufficiently

large to be noticed. In such cases the differentiation might be purely genic, the progenies having the same chromosome number, or the genetic difference might involve a slight change in chromosome number. Even if there was a difference of but one chromosome, the chromosome numbers being for instance 43 and 44, this difference had noticeable morphological consequences. Thesa data certainly indicate that all these chromosomes are genetically active. However, the rather high absolute chromosome number and at least partially autopolyploid constitution will have a buffering effect, diminishing the effects of single genes and chromosomes.

9. *Meiosis and fertility.* — Meiosis in apomicts of *Poa alpina* has not yet been studied sufficiently, but from the evidence available (MÜNTZING, 1932, 1940, and additional data in the present paper; FLOVIK, 1938, p. 316; NYGREN, 1950) we know that in all the biotypes so far examined meiosis is irregular. In some of them complete I-M configurations have been distinguished, showing the presence of univalents, bivalents, trivalents and larger multivalents in various proportions. In two biotypes with 33 chromosomes (from Pajala and Hoting) the number of chromosomes present at meiosis was found to be the same as in the root tips, and, thus, there is at present no reason to assume that accessory chromosomes of the kind observed in a strain of diploid sexual *alpina* (MÜNTZING, 1946, 1948) also occur in the apomicts. In the sexual strain the accessory chromosomes disappear from the root tips but are present at meiosis. They are also smaller than the ordinary chromosomes and more heterochromatic. In the apomicts the somatic chromosomes are not all of equal size and in some biotypes (*cf.* p. 468) they are sufficiently small to be denoted as fragments. Fragments were also found to be present by FLOVIK (1938) in a viviparous biotype from Spitzbergen. As these smaller bodies are present in the root tips, they must be considered to belong to the A-chromosome category rather than to the B-chromosomes. The presence of such fragments may contribute to the formation of aneuploid chromosome numbers, as suggested by RANCKEN (1934), but their presence is probably occasional and does not invalidate the view that we have in *Poa alpina* in most cases a real aneuploidy (*cf.* MÜNTZING, 1940, pp. 179—180).

In spite of the meiotic irregularities pollen fertility in the aneuploids of *Poa alpina* is surprisingly good, but as pointed out previously (MÜNTZING, 1940, p. 178), this is probably a result of gene reduplication caused by autopolyploidy. Previous data as well as new ones reported in the present paper demonstrate that in plants with low chromosome

numbers pollen fertility is less good and pollen size more variable than in plants with higher numbers. Two circumstances may be responsible for this fact. The first one is a stronger buffering effect in plants with high numbers and the second one a more regular meiosis in plants with higher numbers than in those with 33 chromosomes, in which meiosis is, indeed, very irregular.

As pointed out by ÅKERBERG (1941), the good pollen fertility in apomicts of the *Poa* species is also in part the result of natural selection. The species are pseudogamous (*cf.* ÅKERBERG, 1941; HÅKANSSON, 1943) and good pollen quality is therefore a necessary prerequisite for a regular seed formation. In progenies of more or less sexual plants segregants with a greatly reduced pollen fertility are frequently met with. This as well as some other data (*cf.* MÜNTZING, 1940, pp. 129—130) indicates that pollen fertility in *Poa alpina* and *pratensis* is not only influenced by the mode of meiosis and the buffering effect of chromosome reduplication but is also, in some cases, under genetic control.

SUMMARY.

(1) Material of *Poa alpina* from North Sweden and the islands of Öland and Gotland in the Baltic Sea was investigated with regard to chromosome number, morphology and degree of constancy caused by apomictic seed formation or vivipary. Additional data were gathered from a smaller material derived from Norway, Iceland and Greenland.

(2) In the seed-forming types from North Sweden 18 different chromosome numbers were found, ranging from 32 to 50. The numbers 33 and 38 are quite predominating and a third maximum at 44 is also obvious. The Gotland material shows two clear maxima, one at 41 and the other one at 33. In Öland the number 41 is quite predominating and the number 33 quite absent. Instead, there is a small group with 35 chromosomes. The material from Norway, Iceland and Greenland shows a similar range of chromosome numbers as the material from North Sweden. The viviparous biotypes from North Sweden had, on an average, higher chromosome numbers than the seminiferous ones, showing a range from 38 to 56.

(3) The progenies raised were mainly uniform but comprised a low proportion of deviating plants, such aberrants generally being less vigorous than the normal sister plants. The percentages of clearly or possibly aberrant plants were as low as 1,29 for North Sweden, 2,52 for Öland and 1,62 for Gotland. In all cases examined the morphologically

deviating plants were also cytologically deviating, their chromosome number, as a rule, being somewhat lower than in the normal sister plants. In some cases the aberrants were approximately triploid. Progenies of aberrant plants were in some cases more or less constant, in other cases variable. Thus, the aberrants may be more or less sexual but in most cases (in contrast to the conditions in *Poa pratensis*) they are predominantly apomictic.

(4) A morphological study of the material cultivated in the experimental field revealed an enormous morphological diversity with sharp differences between the various progenies. However, especially among material from the same local region cases of morphological identity or similarity between progenies were not infrequent, and in such cases it was possible to establish morphological groups of progenies. Progenies considered to be morphologically identical were also found to have the same chromosome number; progenies differing in chromosome number were always morphologically dissimilar.

(5) The Öland material of *Poa alpina* is characterized by the occurrence of one dominating morphological type with $2n=41$ involving 35 of the 53 progenies observed. Within this group, however, there is much local subdivision. The 35-chromosome plants represent a quite different type.

(6) The 41-chromosome biotypes from Gotland belong to the same main morphological type as most of the Öland biotypes but in striking contrast to them the 41-chromosome progenies from Gotland are widely identical, most of them probably representing exactly the same biotype. The biotypes with 33 chromosomes in Gotland could be subdivided into two well separated groups.

(7) The material from North Sweden could be divided into 19 morphological groups and a number of specific biotypes. Such biotypes are more numerous and, hence, the polymorphism is more pronounced in the alpine region than at subalpine localities and in the lowland. The relative scarcity of different biotypes in the lowland explains cases of identity between progenies from widely different parts of North Sweden. On an average, the alpine biotypes have higher chromosome numbers than biotypes from lower regions.

(8) Additional data on meiotic chromosome associations and pollen fertility are given. A positive correlation between chromosome number and pollen fertility was established. Attempts to correlate chromosome number and various morphological characters gave an almost completely negative result.

(9) Of the conclusions reached in the discussion chapter the following ones seem to be of most interest:

(a) The average degree of apomixis and the average chromosome number in *Poa alpina* are lower in Switzerland and Poland than in Scandinavia.

(b) SKALINSKA's observation that in the Polish mountains the biotypes with the highest chromosome numbers reach the highest altitudes has a counterpart in North Sweden, where the average chromosome number is higher in the alpine region than at lower levels.

(c) As the chromosome numbers of the Swedish *alpina* apomicts are not distributed at random, one or a limited number being dominating in each region, identity in chromosome number between types from different regions indicates a definite relationship.

(d) The 41-chromosome biotypes occurring in Gotland are probably secondarily derived from those occurring in Öland, one or a few of the many biotypes in Öland having emigrated to Gotland at a time when the Ölandic population had already become differentiated into many biotypes.

(e) New biotypes are not only produced through occasional fertilization and production of aberrants with deviating chromosome numbers but probably also through mutation without change in chromosome number. The two processes may be of different importance in different regions. Somatic non-disjunction and elimination may also contribute to the polymorphism.

LITERATURE CITED.

1. BABCOCK, E. B. and STEBBINS, G. L. 1938. The American species of *Crepis*. Their interrelationship and distribution as affected by polyploidy and apomixis. — Carnegie Inst. of Wash., Publ. No. 504, pp. 1—199.
2. BÖCHER, T. W. 1938. Zur Zytologie einiger arktischen und borealen Blütenpflanzen. — Svensk Botanisk Tidskrift, Bd. 32, H. 3, pp. 346—361.
3. BÖCHER, T. W. and LARSEN, K. 1950. Chromosome numbers of some arctic or boreal flowering plants. — Meddelelser om Grønland, Bd. 147, No. 6, pp. 1—32.
4. CLAUSEN, J. 1952. New bluegrasses by combining and rearranging genomes of contrasting *Poa* species. — Proc. of the Sixth Intern. Grassland Congress, pp. 216—221.
5. FAGERLIND, F. 1934. Beiträge zur Kenntnis der Zytologie der Rubiaceen. — Hereditas XIX, pp. 223—232.
6. — 1937. Embryologische, zytologische und bestäubungsexperimentelle Studien in der Familie *Rubiaceae* nebst Bemerkungen über einige Polyploiditätsprobleme. — Acta Horti Berg. 11; 9, pp. 195—470.

7. FLOVIK, K. 1938. Cytological studies of arctic grasses. — Hereditas XXIV, pp. 265—376.
8. GUSTAFSSON, Å. 1947. Apomixis in higher plants. Part II. The causal aspect of apomixis. — Lunds Universitets Årsskrift, N. F., Avd. 2, Bd. 43, No. 2, pp. 71—178.
9. — 1948. Polyploidy, life-form and vegetative reproduction. — Hereditas XXXIV, pp. 1—22.
10. HANCOCK, B. L. 1942. Cytological and ecological notes on some species of *Galium* L., em. SCOP. — The New Phytologist 41, pp. 70—78.
11. HARTUNG, M. E. 1946. Chromosome numbers in *Poa*, *Agropyron*, and *Elymus*. — Am. Journ. Bot., Vol. 33, No. 6, pp. 516—531.
12. HÅKANSSON, A. 1943. Die Entwicklung des Embryosacks und die Befruchtung bei *Poa alpina*. — Hereditas XXIX, pp. 25—61.
13. LÖVE, A. and LÖVE, D. 1949. The geobotanical significance of polyploidy. 1. Polyploidy and latitude. — Portugaliae Acta Biologica, Series A. — R. B. GOLDSCHMIDT Volumen, pp. 273—352.
14. MELCHERS, G. 1946. Die Ursachen für die bessere Anpassungsfähigkeit der Polyploiden. — Zeitschr. f. Naturforsch. 1, pp. 160—165.
15. MÜNTZING, A. 1932. Apomictic and sexual seed formation in *Poa*. — Hereditas XVII, pp. 131—154.
16. — 1936. The evolutionary significance of autopolyploidy. — Hereditas XXI, pp. 263—378.
17. — 1940. Further studies on apomixis and sexuality in *Poa*. — Hereditas XXVI, pp. 115—190.
18. — 1946. Different chromosome numbers in root tips and pollen mother cells in a sexual strain of *Poa alpina*. — Hereditas XXXII, pp. 127—129.
19. — 1948. Accessory chromosomes in *Poa alpina*. — Heredity, Vol. II, Part I, pp. 49—61.
20. — 1949. Accessory chromosomes in *Secale* and *Poa*. — Proc. of the Eighth International Congress of Genetics (Hereditas, Suppl. Vol., 1949, pp. 402—411).
21. NANNFELDT, J. A. 1940. On the polymorphy of *Poa arctica* R. BR., with special reference to its Scandinavian forms. — Symbolae Botanicae Upsalienses IV: 4, pp. 1—85.
22. NYGREN, A. 1949 a. Studies on vivipary in the genus *Deschampsia*. — Hereditas XXXV, pp. 27—32.
23. — 1949 b. Apomictic and sexual reproduction in *Calamagrostis purpurea*. — Hereditas XXXV, pp. 285—300.
24. — 1950. Cytological and embryological studies in arctic *Poae*. — Symbolae Botanicae Upsalienses X: 4, pp. 1—64.
25. RANCKEN, G. 1934. Zytologische Untersuchungen an einigen wirtschaftlich wertvollen Wiesengräsern. — Acta Agralia Fennica 29, pp. 1—92.
26. SKALINSKA, M. 1949—50. Studies in chromosome numbers of Polish Angiosperms. — Acta Societatis Botanicorum Poloniae, Vol. XX, No. 1, pp. 45—68.
27. — 1952. Cyto-ecological studies in *Poa alpina* L. var. *vivipara* L. — Bull. de l'Académie Polonaise des Sciences et des Lettres, 1951, Série B, pp. 253—283.
28. SOKOLOVSKAJA, A. P. and STRELKOVA, O. S. 1938. Polyploidy in the high mountain regions of Pamir and Altai. — Comptes Rendus (Doklady) de l'Académie des Sciences de l'URSS, Vol. XXI, Nos. 1—2, pp. 68—71.

29. TISCHLER, G. 1954. Allgemeine Pflanzenkaryologie. — Handbuch der Pflanzenanatomie, Band II. Ergänzungsband: Angew. Pflanzenkaryologie, Zweite Lief., pp. 209—416.
30. TURESSON, G. 1927. Contributions to the genecology of glacial relics. — Hereditas IX, pp. 81—101.
31. — 1930. Studien über *Festuca ovina* L. II. Chromosomenzahl und Viviparie. — Hereditas XIII, pp. 177—184.
32. — 1931. Studien über *Festuca ovina* L. III. Weitere Beiträge zur Kenntnis der Chromosomenzahlen viviparer Formen. — Hereditas XV, pp. 13—16.
33. ÅKERBERG, E. 1939. Apomictic and sexual seed formation in *Poa pratensis*. — Hereditas XXV, pp. 359—370.
34. — 1941. Cytogenetic studies in *Poa pratensis* and its hybrid with *Poa alpina*. — Hereditas XXVIII, pp. 1—126.

CONTENTS.

I. Introduction 459
II. Chromosomal variation 460
III. Constancy and formation of aberrant plants 463
1. Frequency of aberrants 463
2. Chromosome numbers and vigour of the aberrant plants 464
3. Progenies of aberrant plants 466
A. Chromosome numbers of aberrant plants somewhat reduced. Progenies with poor vigour but predominantly constant 466
B. Chromosome numbers of aberrants somewhat reduced. Progenies poor and variable 467
C. Aberrants probably triploid giving more or less constant progenies 467
D. Two other cases 468
IV. Occurrence of biotype groups 468
1. Introduction 468
2. Biotype groups in Öland 469
A. The 41-chromosome main type 470
B. Other morphological groups 471
C. Conclusions concerning the Öland material 472
3. Biotype groups in Gotland 473
A. Biotypes with 41 chromosomes 473
B. Biotypes with 33 chromosomes 474
C. Some other biotypes 475
D. Conclusions concerning the Gotland material 475
4. Biotype groups in North Sweden 476
5. Cases of identity between progenies from widely separated localities ... 479
V. Degree of polymorphism in different groups of material 481
A. Biotypes from the lowland 481
B. Biotypes from subalpine localities 481
C. Biotypes from the alpine region in South and Middle Lapland 482
D. Biotypes from the alpine region in North Lapland 483

VI. Chromosome numbers in different groups of material from North Sweden 486
1. Seed-forming biotypes 486
2. Viviparous biotypes 488
VII. Some data concerning material from Norway, Iceland and Greenland 489
VIII. Meiotic observations 491
IX. Pollen fertility 492
X. Chromosome number and morphology 493
XI. Discussion 495
1. Degree of apomixis in various biotypes of *Poa alpina* 495
2. Nature of aberrants and their mode of reproduction 496
3. Chromosome numbers in the Swedish material 498
4. Chromosome numbers in material from other countries 499
5. Chromosome number and ecology 501
6. Degree of polymorphism in different areas. Biotype groups 504
7. Formation of new biotypes 506
8. Chromosome number and morphology 508
9. Meiosis and fertility 510
Summary 511
Literature cited 513

ÜBER HOCHKOMPLEXE BASTARDVERBINDUNGEN IN DER GATTUNG SALIX

VON *HERIBERT NILSSON*
BOTANISCHES INSTITUT, LUND, SCHWEDEN

MEINE Bastardierungsversuche mit *Salix* begannen im Jahre 1906 (HERIBERT NILSSON, 1918), sind also bald 50 Jahre gelaufen. Ich habe dabei nicht nur analytisch, sondern auch synthetisch gearbeitet.

Über den sukzessiven Aufbau eines septenären Produkts habe ich schon 1937 berichtet. Dieses war folgendes:

Salix

$$\left\{\begin{array}{r}(\textit{purpurea}\ \text{L.}\times\textit{daphnoides}\ \text{VILL.})\\ \times\\ (\textit{repens}\ \text{L.}\times\textit{aurita}\ \text{L.})\end{array}\right\}$$
$$\times$$
$$\left\{\begin{array}{r}(\textit{phylicifolia}\ \text{L. s. lat.}\ \textit{Weigeliana}\times\textit{ph. nigricans})\\ \times\\ (\textit{viminalis}\ \text{L.}\times\textit{caprea}\ \text{L.})\end{array}\right\}$$

Ich habe diese hochkomplexe Bastardpopulation *S. polygena* genannt. Von ihrem Aufbau, ihren Eigenschaften und ihrem artbildungstheoretischen Wert habe ich in meiner neulich erschienenen Arbeit über das Artbildungsproblem (HERIBERT NILSSON, 1953) ausführlich berichtet.

Den *polygena*-Komplex habe ich zuerst (1937) eine oktonäre Verbindung, später (1953) eine septenäre genannt. Die Ursache ist, dass ich die vieldebattierten Arten *S. phylicifolia* L. s. str. (*S. Weigeliana* WILLD.) und *S. nigricans* SM. (*S. phylicifolia* β L.) als eine einzige Spezies, *S. phylicifolia* L. s. lat., betrachten muss (HERIBERT NILSSON, 1953, pp. 256—260). Über die verwendete ternäre Benennung der Teilpopulationen der Spezies habe ich in angegebener Arbeit (pp. 340—342 und 352—355) die Motivierung gegeben.

Mein Bestreben ist während der letzten Jahrzehnte gewesen, noch weitere Komplexverbindungen darzustellen. In der betreffenden Arbeit konnte ich schon von einer gelungenen Komplexverbindung von 13 Spezies berichten und zwar:

$$\left\{\left\{\begin{array}{c} \textit{polygena} \text{ (aus 7 Spezies)} \\ \times \\ (\textit{silesiaca} \times \textit{aegyptiaca}) \\ \times \\ (\textit{grandifolia} \times \textit{gracilistyla}) \end{array}\right\} \times (\textit{cinerea} \times \textit{glabra})\right\}$$

Von dieser Population habe ich im Versuchsgarten 13 Pflanzen. Die Mehrzahl hat in bezug auf die Blattform den in Fig. 1, rechts, angegebenen Typus. *S. cinerea* und *glabra* prägen das Aussehen am deutlichsten, dazu *grandifolia*. Das ist indessen auch natürlich, denn der oktonäre Komplex ist ja zur Hälfte von Genen der erstgenannten Spezies aufgebaut. Die neuen Bastarde *silesiaca*×*aegyptiaca*, *grandifolia* ×*gracilistyla* and *cinerea*×*glabra* sind, wie das Schema angibt, nicht wie ein neuer 6-Bastard mit *polygena* gekreuzt, sondern sind *sukzessiv* diesem Komplex zugefügt. Die Gene der 7 *polygena*-Stammarten sind also dreimal stark zurückgedrängt worden. Dass sich Merkmale dieser Arten stärker sichtbar machen sollten, ist deshalb kaum zu vermuten. Die grossblättrigen Arten der drei letzten Einkreuzungen, vor allem *aegyptiaca*, *grandifolia* und *glabra*, bewirken ein Aussehen, das nicht gering eine Teilnahme von *caprea* in dem Endprodukt vortäuscht.

Die tredezenäre Kreuzung wurde 1950 ausgeführt. Drei verschiedene Verbindungen wurden zwischen Sträuchern des undezenären Komplexes, von dem ich 81 Sträucher hatte, und *cinerea*×*glabra*, die 44 Sträucher enthielt, ausgeführt. In sämtlichen Fällen war der Samenansatz recht gut, aber die Samen waren von sehr differenter Keimfähigkeit und Keimkraft. Zum Auspflanzen im nächsten Jahr blieben nur 13 Pflanzen übrig.

Diese sind nun nach vier Jahren gut gewachsen. Vier Sträucher sind klein, drei sehr kräftig, deutlich heterotisch, die übrigen mittelkräftig. In bezug auf den habituellen Typus repräsentieren sie eine ganz besondere Population. Zwei Extremtypen betreffs der Vitalität sind in Fig. 1, *a* und *b*, zu sehen. Die Mehrzahl der 13 Sträucher ähnelten in bezug auf die Blattform dem in der Fig. 1 *a* dargestellten Typus. Sie waren oval, rundlich bis verlängert, in einigen Fällen aber schmäler, elliptisch oder verkehrt eiförmig. Im ersten Falle erinnern sie am meisten an die Arten *caprea*, *grandifolia* und *glabra*, im letzteren an *cinerea* und *gracilistyla*. Die Gene der 7 *polygena*-Arten, ausser *caprea*, sind kaum habituell zu bemerken. Das ist ja nicht zu verwundern.

b *a*

Fig. 1. Zwei Extremtypen in bezug auf die Blattform und die Vitalität des tredezenären Bastards.

Denn die neuen Bastarde, *silesiaca*×*aegyptiaca*, *grandifolia*×*gracilistyla* und *cinerea*×*glabra*, sind — wie schon oben erwähnt — *sukzessiv* mit dem *polygena*-Komplex gekreuzt worden. Die Gene der 7 *polygena*-Stammarten sind deshalb dreimal stark zurückgedrängt worden. Denn bei der wiederholten Kreuzung zwischen Komplexbastard×neuem binären Bastard trägt ja der letztere zur Hälfte mit den Gameten bei, während die sieben *polygena*-Arten nur *zusammen* zur anderen Hälfte mit Spaltungsgameten beitragen. Bei jeder wiederholten Kreuzung fin-

det eine derartige »Auswässerung» des *polygena*-Komplexes statt. Habituell war von den Arten dieser Verbindung: *aurita*, *daphnoides*, *phylicifolia*, *purpurea*, *repens* und *viminalis* nichts zu sehen.

Die Ähnlichkeit mit *caprea* kann eigentümlich vorfallen, denn diese Art ging schon in dem *polygena*-Komplex ein, dürfte deshalb genisch sehr depauperiert sein. Da ich früher gezeigt habe, dass *cinerea* als amphidiploid aus *viminalis*×*caprea* hervorgegangen ist (HERIBERT NILSSON, 1931), sind *caprea*-Gene wirklich von Neuem in dem Endkomplex eingeführt worden.

Kein Strauch hatte ganz kahle Blätter. Die Rippe war immer behaart, ebenso der Blattstiel, ober- und unterseits. Die Spreite war längs der Rippe behaart, aber dann ± kahler werdend, und gegen den Blattrand war sie immer ganz kahl.

Die Haare der Blattunterseite waren ± *vorwärtsgerichtet*, ganz wie bei *phylicifolia nigricans*. Diese Spezies geht ja zwar in *polygena* ein, aber es war kaum zu erwarten, dass ihre Behaarung sich so stark in dem Endprodukt geltend machen könnte. Der Charakter stammt auch nicht aus dem *nigricans*-Typus der *phylicifolia*, sondern aus einer der zuletzt eingekreuzten Arten, nämlich *gracilistyla*, die strikt vorwärtsgerichtete Haare der Blattunterseite hat.

Die Rippe war bei sämtlichen Sträuchern ± rötlich gefärbt. Da sowohl *silesiaca* wie auch *aegyptiaca* und *grandifolia* rötlich oder rötlich angehauchte Rippen haben, ist das verständlich. Denn diese Arten gehören alle zu den zuletzt eingekreuzten.

Der Blattrand war bei der Mehrzahl der Sträucher, besonders der grossblättrigen, kerbig bis bogig oder gross ausgebissen gesägt, also immer von auffallender und unregelmässiger Zerschlitzung. In dieser Eigenschaft erinnerte der Komplexbastard am meisten an *aegyptiaca* und *silesiaca*.

Die Mehrzahl der Sträucher hatte keine oder sehr kleine Nebenblätter; nur bei zwei Individuen kamen sie wohl ausgebildet vor.

Eine Eigenschaft, die für die *cinerea*-Gruppe sehr charakteristisch ist, ist die am Holz vorkommenden Striemen. Von den drei Sträuchern, die so wohlentwickelt waren, dass diese Eigenschaft untersucht werden konnte, zeigten sämtliche Striemen auf. *S. cinerea* war ja auch eine der zuletzt eingekreuzten Arten.

Die Hälfte der Sträucher hat schon geblüht. Alle waren ♀. Ich habe versucht, noch einen binären Bastard, nämlich *integra* THUNB.× *incana* SCHRANK, in dem tredezenären Produkt einzukreuzen. Bis jetzt ist es wegen der offenbar starken, vielleicht vollständigen Sterilität

dieses, misslungen. Von *integra*×*incana* habe ich indessen mehrere ♂-Sträucher, die noch nicht untersucht sind. Und die Kreuzung *cinerea*× *glabra* (also der letzte Zufügungsbastard des Tredezenären) habe ich mit *integra*×*incana* kreuzen können. Es ist deshalb wohl nicht ganz aussichtslos, in angegebener Weise einen quindezenären Bastard erhalten zu können.

Sehr viel interessanter wäre indessen, durch zwei quaternäre Bastarde ein oktonäres Produkt ganz anderer Arten als die, welche in *polygena* eingehen, aufzubauen zu versuchen und dann diesen Komplex mit *polygena* zu kreuzen, also nicht sukzessiv, sondern so »schnell» wie möglich einen hochgenischen Komplex aufzubauen.

Das Fertigstellen einer derartigen oktonären Kreuzung ist mir schon gelungen. Diese ist

$$\left|\begin{matrix}(cinerea \times glabra)\\ \times \\ (integra \times incana)\end{matrix}\right| \times \left|\begin{matrix}(grandifolia \times silesiaca)\\ \times \\ (aegyptiaca \times Reinii)\end{matrix}\right|$$

Von den in diesem Komplex eingehenden Arten ist nur eine schwedisch und europäisch, nämlich *cinerea*. *S. grandifolia* SER., *silesiaca* WILLD., *incana* SCHRANK und *glabra* SCOP. sind mitteleuropäisch, *aegyptiaca* L. ist südwestasiatisch (FLODERUS, 1933), *gracilistyla* MIQUEL, *integra* THUNB. (*amplexicaulis* CHAUB., *multinervis* FRANCH. et SAV.) und *Reinii* FRANCH. et SAV. sind alle ostasiatisch (Kina, Korea, Japan).

In bezug auf die zytologischen Verhältnisse sind die Arten *glabra*, *gracilistyla*, *grandifolia*, *incana* und *silesiaca* normal diploid ($n=19$). *S. Reinii* hat sich als oktoploid erwiesen, *aegyptiaca* als tetraploid (HÅKANSSON, unpubliziert). Über die tetraploide (amphidiploide) *S. cinerea* habe ich früher berichtet (HERIBERT NILSSON, 1931). *S. integra* ist noch nicht untersucht.

Die oktonäre Kreuzung der beiden quaternären Komplexe wurde 1952 durchgeführt. Der Samenansatz war recht gut, aber die Mehrzahl der Samen war klein, bleich, schon dem Aussehen nach von geringer Vitalität und kaum entwicklungsfähig. Nur vereinzelte waren wohlausgebildet, grün. Für die Kreuzung hatte ich 5 Kätzchen von je 3 Zweiglein gekapselt. Da ein Kätzchen ca. 50 Blüten enthält und da

jede Kapsel gewöhnlich mehr als 1 Samenanlage hat, wurden mehr als 1000 Samenmöglichkeiten geprüft.

Von den scheinbar guten, grünen Samen entstanden mehrere Pflanzen. Während des Vorsommers begannen sie, kaum noch 1 cm hoch, zu vergilben. Zuletzt blieb nur eine einzige Pflanze übrig, die weiter wuchs. Da auch diese im Hochsommer ein gelbliches Aussehen annahm, führte ich ihr Nährlösung oft und jedesmal spärlich zu. Sie erholte sich und hatte im Herbst eine Höhe von 1 dm. Sie überwinterte gut und ist nun (August 1954) eine kräftige, verzweigte, 30 cm hohe Pflanze.

Die Aussicht, dass es mir gelingen wird, einen Bastard aus 15 *Salix*-Spezies herzustellen, scheint also recht gut zu sein. Auch falls der oktonäre Bastard ein ♀-Strauch wird, wie in den Komplexverbindungen sehr gewöhnlich, so liegen von der septenären *polygena* schon ♂-Sträucher vor.

ZITIERTE LITERATUR.

1. Floderus, B. 1933. *Salix aegyptiaca* L. — Arkiv för Bot. 25 A, Nr. 11.
2. Hao, Kin-Chen. 1936. Synopsis of Chinese *Salix*. — Berlin.
3. Nakai, T. 1930. *Salicaceae*. Flora Sylvatica Koreana. Pars 18. — Keijijo.
4. Nilsson, Heribert. 1918. Experimentelle Studien über Variabilität, Spaltung, Artbildung und Evolution in der Gattung *Salix*. — Lunds Univ. Årsskr., N. F., Avd. 2, Bd 14, Nr 28.
5. — 1931. Über das Entstehen eines ganz *cinerea*-ähnlichen Typus aus dem Bastard *Salix viminalis*×*caprea*. — Hereditas XV.
6. — 1937. Ein oktonärer, fertiler *Salix*-Bastard und seine Deszendenz. — Hereditas XXII.
7. — 1953. Synthetische Artbildung. Grundlinien einer exakten Biologie. I—II. — Lund, C. W. K. Gleerups Förlag. 1303 pp.
8. Toepffer, Ad. 1915. *Salices* Bavariae. — München.

ABSTRACTS

ARTUR HÅKANSSON: Transmission of accessory chromosomes in *Poa alpina*.

Accessory chromosomes were detected in *Poa alpina* by MÜNTZING who has described their appearance and behaviour in detail (MÜNTZING, 1946, 1948). They occurred in a certain sexual strain with the diploid chromosome number ($2n=14$). The accessory chromosomes were considerably smaller than the ordinary ones, and they had a median centromere. MÜNTZING established certain interesting peculiarities. Most accessory chromosomes are heterochromatic; in *Poa alpina*, however, they showed only certain "symptoms of being heterochromatic" (compare also HÅKANSSON, 1948). At diakinesis and first metaphase they never formed multivalents, but only bivalents or/and univalents. As also observed in *Sorghum purpureo-sericeum*, the accessories seem to be restricted to what may be termed the "germ-line" of the plant: they occurred in pollen mother cells but were absent in roots. Later it was found that they are eliminated in young leaves, too (MÜNTZING, 1949). In contradistinction to *Sorghum* the accessories are present in the primary root of the young plant; only rarely one or two accessories were lost during the development of the embryo, mostly in the primary root during the last division before the resting stage (HÅKANSSON, 1948).

The number of accessories was rather high, MÜNTZING counted 2—8 in 34 plants. Plants having an even number were more frequent than plants with an odd number. These facts suggested to MÜNTZING an accumulation mechanism tending to increase the number of accessories. Such a mechanism has been found in different grasses in the form of non-disjunction of accessories at the first, or more rarely the second, pollen mitosis (*Secale cereale*, *Festuca pratensis*, *Anthoxanthum aristatum*, *Zea mays*; in *Secale* also at the first mitosis in the embryo-sac). In *Poa alpina* the first pollen mitosis is regular; MÜNTZING, therefore, assumed a somatic doubling, probably a non-disjunction involving all accessories.

In the female line the accessory chromosomes of *Poa alpina* show a similar behaviour as in the anthers, nor were peculiarities observed during the development of the embryo (HÅKANSSON, *l. c.*).

I have tried to determine the transmission of accessory chromosomes to the progeny, counting the chromosomes in what may be called parental plants and in hybrids between parental plants with a known number of accessories. The material has been put to my disposal by Professor MÜNTZING. This has been a rather time consuming task, partly owing to the difficulty of obtaining good fixations. In most cases MÜNTZING counted the chromosomes at the first anaphase in the pollen mother cell, I have more often used the diakinesis stage. The latter stage occurs much more often in the slides and may be sufficiently clear to allow exact counts. A difficulty is that the number of accessory chromosomes may vary within the individual. MÜNTZING (1948) stresses that the number of accessories is fairly constant but sometimes may vary within the individual not only in different years and in different panicles but also within the same anther loculus.

I have determined the chromosome number in 23 parental plants while in other such plants no exact counts were possible. The number of accessory chromosomes was on an average lower than in the plants investigated by MÜNTZING. However, the investigated material belonged to different generations, the plants now studied being daughters of the plants investigated by MÜNTZING.

Number of accessories	2	3	4	5	6	7	8
Parental plants	3	6	11[1]	1	2	—	—
Exact counts by MÜNTZING	2	1	5	2	6	—	2
Approx. counts by MÜNTZING	1	1	6	2	5	1	2

[1] In two plants also 5 were counted.

The rather high number of plants with three accessories was unexpected. The numbers found scarcely speak in favour of an accumulation of accessory chromosomes.

Hybrid plants were obtained from eight different reciprocal crosses where the chromosome number of the two parental plants were known. The result of the counts is listed in Table 1. Exact counts could be made in 65 hybrid plants.

Table 1. *Number of accessories in hybrid plants.*

		2	3	4	5	6
Cross 1	3×4		3	1		1
	4×3		1			
Cross 2	4×2		6	1		
	2×4		2			
Cross 3	2×4		2			
Cross 4	3×4		3	4		
	4×3	2[1]	2	1		
Cross 5	3×4		1	2		
	4×3		1			1
Cross 6	4×6				3	
	6×4				4	2
Cross 7	4×5			3	3	
	5×4			2	3[2]	
Cross 8	4[a]×4			3	3	
	4×4[a]			3		3

[1] Also 3 accessories. — [2] 2 plants also 6. — [3] Also 5.

In others the number was uncertain. However, in all investigated parental and hybrid plants accessory chromosomes were present; so far, no plant belonging to this strain of *Poa alpina* lacking accessories has been found.

Evidence of an increase in number of accessories is absent or very weak. A generally functioning accumulation mechanism cannot occur. Plants with an "unexpected" number were few. Exceptions with one chromosome more or less

than the expected number are probably the result of non-disjunction of accessories at first anaphase in a parental plant. Irregular distribution at this stage is not unfrequent owing to the fact that accessories sometimes are unpaired. Another cause may be variation in number of accessories among the pollen mother cells of the parental plants.

Only in cross 5 and in cross 1 we find a plant with a chromosome number suggesting a doubling of all accessories (from 3 to 6).

Cross 8 showed an unexpectedly high number of accessories in several plants. One parental plant had, however, an oscillating number which also was observed in certain plants in the progeny.

Thus, several instances of intra-individual variation in number of accessories have been found also in this investigation. The observations by MÜNTZING on the behaviour of the accessories during meiosis have been confirmed.

Figs. 1—3. Accessory chromosomes at diakinesis. — 1: plant with 3 access. II+I, 3 I and III. — 2: plant with 4 access. IV's. — 3: plant with 5 access. IV+I and V. — Multivalents are very rare, as a rule bivalents are formed.

At diakinesis the accessories form small bivalents with one or two terminal chiasmata. An interstitial chiasma was never observed in small bivalents though it may occur in larger bivalents. MÜNTZING stresses that small bivalents have a tendency to stick together, but that a forming of true multivalents never occurred. The ability to form multivalents is possibly not quite absent. Figs. 1—3 show some instances of probable multivalents. The absence of interstitial chiasmata makes, however, a sure conclusion difficult; it may be cases of stickiness. Plants with an odd number of accessories generally show a univalent at diakinesis; rarely a nucleus with three accessories has three univalents. At first metaphase unpaired accessories seem more frequent. As has been shown previously, they go undivided to the pole at anaphase while large univalents divide. Only very exceptionally a division of a small univalent was observed.

The first anaphase may show irregularities. In some fixations "lagging" was frequent but seems in most cases not to result in chromosome loss, belated chromosomes being included in telophase nuclei (compare also MÜNTZING). Irregular distribution (non-disjunction), for instance, 3+1 instead of 2+2 may occur when accessories in exceptional cases are unpaired.

The behaviour of the accessories during meiosis seems sufficient to explain their transmission, though exceptional cases of doubling seem to occur. The failure to find any plant lacking accessories does perhaps not depend on chance but on the inviability of such plants (or gametes?).

Institute of Genetics, Lund, Sweden.

Literature cited.

1. HÅKANSSON, A. 1948. Embryology of *Poa alpina* plants with accessory chromosomes. — Hereditas XXXIV.
2. MÜNTZING, A. 1946. Different chromosome numbers in root tips and pollen mother cells in a sexual strain of *Poa alpina*. — Hereditas XXXII.
3. — 1948. Accessory chromosomes in *Poa alpina*. — Heredity II.
4. — 1949. Accessory chromosomes in *Secale* and *Poa*. — Proc. 8th Int. Congr. Genetics (Hereditas, Suppl. Vol.).

E. J. OLDÉN: Giant pollen grains in fruit trees from colchicine treatment in vacuum.

At the Balsgård Fruit Breeding Institute experiments with polyploidy induction have been performed since 1943, shortly after the foundation of the institute. Seeds and vegetative shoots have been treated with colchicine, and a series of chimaerical polyploids have been obtained in various fruit tree species but also a few cases of complete genome doubling. Among these latter types a pure tetraploid *Prunus avium* can be mentioned, obtained by Mrs. BERTA DANIELSSON-SANTESSON (unpubl.) after colchicine treatment (1 per cent solution) of germinating seeds.

In the late winter of 1953 the present author started some colchicine treatments with fruit buds of different *Prunus* species in order to get pollen grains with higher chromosome number for crossing purposes.

Investigations on the effect of colchicine on meiotic chromosomes were first reported by WALKER (1938) and DERMEN (1938) in *Tradescantia* and *Rhoeo*. In 1939 LEVAN published his studies on colchicine meiosis in *Allium* (1939 a). He immersed whole inflorescenses in solutions of different concentrations; especially in 1 per cent colchicine he observed the formation of pollen chambers containing uninucleate pollen grains of dyad or monad type. In sugar beets RASMUSSON and LEVAN (1939) reported pollen grains of different size from colchicine treatment of stamens.

In the fruit trees, however, the dipping methods used by the above-mentioned authors seem very difficult to apply with good results. The fruit buds are often so densely closed by the bud scales during the meiotic division in the P.M.C's that the uptake of the solution is inconsiderable. The removing of the bud scales will easily damage the flower buds (the young flowers in the fruit bud) and stop their development. Attempts were therefore made to inject the solution in cherry fruit buds with a thin syringe-needle. The damage caused, however, that nearly all of the treated buds failed to develop. In the investigated surviving flowers only one single anther with giant pollen grains could be found. In diploid plums the injection experiments were out of question because of the small size of the fruit buds.

At the end of February, 1953 a vacuum technique was applied on pot trees of plums and cherries in a glasshouse. The idea was to obtain a more uniform colchicine penetration of the buds. In *Petunia* seeds LEVAN (1939 b) has tried this method; he reports, however, that the lethality was very high (only 93

surviving plants from about 2000 seeds). In the experiments of the present author shoots with fruit buds of the appropriate stage were covered by test tubes and one at a time inserted into a vacuum pump glass shade through the top tube. Round the shoot an effective tightening was achieved by means of rubber stopper and paraffin. The test tube was nearly filled with 1 or 1 1/2 per cent colchicine solution. The pressure in the shade was kept at 18—45 mm Hg for 5—10 minutes, the best results being obtained at 25—35 mm Hg. Many air bubbles were seen coming from the lenticels of the bark but also from the tips of the buds. Wounds on the twigs were covered with melted paraffin to avoid too much air leakage. When the normal air pressure had been restored after the vacuum the shoots were left in the colchicine solution for 5 minutes to 24 hours. In the flower buds the best uptake was found at 1—4 hours' after-treatment. In the leaf buds the longest time, 24 hours, gave the best results. All fruit buds were, however, killed or severely damaged in their P.M.C's during this long treatment, especially in the stronger solution of 1 1/2 per cent. The developmental stage and the result of treatment were controlled in aceto-carmine stains.

In February and March this year a new series of experiments were carried out with pot trees of several varieties of diploid plums and sweet and sour cherries. Plum twigs of easily forced varieties were also taken from trees in the orchard. The vacuum was kept between 25—35 mm Hg in accordance with the experience of the preceding year. The plums were treated for 7—8 minutes in 1 or 1 1/2 per cent colchicine solution at this low pressure; for the cherries the time was extended to 10 minutes. In the 1 per cent solution the shoots had to stay 3 to 4 hours after the vacuum, in the 1 1/2 per cent solution only 1—2 hours. In most experiments the treatment was given to anthers in the reduction division stage but in some cases to very young P.M.C's and archespores.

The investigations of the anthers after the pollen formation indicated that the best results had been obtained by treatment during the reduction division stage, and that the treatment at earlier stages gave a very high proportion of aborted P.M.C's. In flowers where giant pollen grains were found often half the number of anthers contained only normal haploid grains. The reason seems to be that the stamens belong to different development stages. In stone fruit flowers the inner circles of stamens develop earlier than do the outer ones (contrary to the case in apples and pears). However, a few flowers of the plum variety Beauty had giant pollen grains in all their anthers. Some of these anthers had one theca with pollen grains of diploid type whereas the other theca contained grains of presumably tetraploid constitution. It should be noted that in many fruit buds all flowers carried normal pollen after the treatment, which may depend upon too late treatment or too small colchicine uptake.

The purpose of the treatments was to produce giant pollen grains for cross experiments. Since most flowers in question contained anthers with giant pollen grains as well as normal ones it was necessary to make a selection. If mixtures of normal and giant pollen grains are used for pollination probably the pollen tubes of the haploid grains will grow faster in the style than those of the giant ones. It was quite easy to carry out the selection in a preparation microscope magnifying about 30 times. In another microscope magnifying about 70 times a control of the selected and burst thecae could be performed. Especially good

results were obtained with the plum varieties Beauty, Burbank, Santa Rosa, and 0—3010. In these varieties more than 50 per cent of the flowers had one or many stamens with giant pollen. Similar results were reached in the sweet cherry Heinrichs Riesen. Other varieties with giant pollen grains in many anthers were Frogmore, Allmän Gulröd Bigarrå and Ostheimer.

The styles of the treated flowers were usually dead or severely damaged. They seemed to be more sensitive than the stamens. In flowers with normal pistil no colchicine effect could be found in the anthers.

Some pollen germination tests were made in 15 per cent cane sugar solution. No disturbances in the germination of the giant pollen grains were observed. Even after 4 hours these grains presented just as good pollen tubes as did the normal haploid grains.

The pollination experiments with giant pollen grains in 1953 have resulted in some seedlings with higher chromosome number. The diploid cherry variety Napoleon was fertilized by giant pollen of the colchicine treated diploid cherry Rivers Early. When the fruits were going to ripe the embryos were prepared for cultivation in a nutritional solution according to the method proposed by TUKEY (1934). Among 17 investigated seedlings 3 plants with the triploid chromosome number ($2n=24$) were found. In plums only a few seedlings developed, all having the normal diploid number.

This year a great many pollinations have been carried out. Judging from the fruit set good results can be expected in plums as well as cherries. Experiments with apples have also been started this year, and Mr. ANJOU (unpubl.) reports that giant pollen grains have been found in the diploid varieties Cox's Orange, Cox's Pomona and Ingrid Marie.

The method seems to offer some new prospects in the breeding programme. It is usually presumed that the Duke cherries ($2n=32$) have originated spontaneously by the fertilization of *Prunus cerasus* ($2n=32$) with unreduced diploid pollen from *P. avium* ($2n=16$). This hypothesis will now be tested in experiments. Another aspect is to produce new tetraploid *P. avium* by fertilizing the available, highly sterile types with giant pollen from the best market varieties of sweet cherries. In apples and pears it will be possible to obtain new triploids and tetraploids from the best varieties and types. In the treatments of apples some triploids are consequently also included in order to produce triploid pollen grains for these pollination experiments.

Balsgård Fruit Breeding Institute, Fjälkestad, Sweden.

Literature cited.

1. DERMEN, H. 1938. A cytological analysis of polyploidy induced by colchicine and by extremes of temperature. — Journ. of Hered. 29: 211—220.
2. LEVAN, A. 1939 a. The effect of colchicine on meiosis in *Allium*. — Hereditas XXV: 9—26.
3. — 1939 b. Tetraploidy and octoploidy induced by colchicine in diploid *Petunia*. — Hereditas XXV: 109—131.
4. RASMUSSON, J. and LEVAN, A. 1939. Tetraploid sugar beets from colchicine treatment. — Hereditas XXV: 97—102.

5. TUKEY, H. B. 1934. Artificial culture methods for isolated embryos of deciduous fruits. — Proc. Amer. Soc. Hort. Sci. 32: 313—322.
6. WALKER, R. I. 1938. The effect of colchicine on microspore mother cells and microspores of *Tradescantia paludosa*. — Am. Journ. of Bot. 25: 280—285.

S. FRÖST: The genetic effect of accessory chromosomes in *Centaurea scabiosa*.

Accessory chromosomes (also called B or supernumerary chromosomes) have been found in a large number of species both in plants and animals (for a review of the botanical literature on this subject, see TISCHLER, 1953).

Among plants accessory chromosomes have been most extensively studied in rye and maize. In these two species a detailed cytological study of their behaviour and structure as well as an analysis of their transmission has been made. For rye, see MÜNTZING, 1944, 1945, 1946, 1948; HÅKANSSON, 1948; MÜNTZING and LIMA-DE-FARIA, 1949, 1952; and for maize, DARLINGTON and UPCOTT, 1941; RANDOLPH, 1941; ROMAN, 1947, and others.

Data on the genetic effect of accessory chromosomes are more scanty. In a study on the genetic effect of accessory chromosomes in rye MÜNTZING (1943) found a negative effect on vegetative development and fertility. The effect on the vegetative development was non-linear; the effect of high numbers of accessory chromosomes being comparatively stronger. The same negative effect has also been found in maize (RANDOLPH, 1941).

The genetic effect of accessory chromosomes in *Centaurea scabiosa* has been studied in a similar way as in rye and maize.

A study of the transmission of the accessory chromosomes in *Centaurea* (data given by FRÖST in MÜNTZING, 1954) as well as direct cytological observations have proved the existence of a certain degree of meiotic elimination in most plants. As there is no evidence of a mechanism for numerical increase of the accessories in this species, as is the case especially in the grasses, *e. g.*, *Secale, Zea, Anthoxanthum*, and *Festuca*, it seems likely that at least under some conditions the meiotic elimination is compensated by natural selection for plants having accessory chromosomes. If this were not the case it would be difficult to understand how they can maintain themselves in natural populations. Studies of the geographical distribution of plants with accessories have shown that especially in some geographical areas accessory chromosomes are very common (data given by FRÖST in MÜNTZING, 1954).

To test an eventual positive effect of accessory chromosomes a series of experiments were carried out: in the field as well as in a greenhouse. Characters such as plant weight, fertility, earliness and germination capacity were studied.

All the results now reported concern the effect of a *low number of accessory chromosomes*. The low numbers, being most common in natural populations, are consequently of special interest to investigate. (Out of 1700 plants with accessory chromosomes from 92 natural populations about 72 per cent had only 1, 2 and 3 accessories.)

Field experiments. — The material for these experiments consists of the progenies from five crosses of the type (20+1 acc.)×(20+1 acc.), originating

from two male sterile mother plants which were siblings. Of the five paternal parents, which were not related, three were crossed with one mother plant and two with the other. The crosses were made in the following way: With the aid of a brush the pollen was applied to the receptive stigmatic surfaces of the flowers selected as mothers. All heads used as maternal as well as paternal parents had previously been isolated well before flowering. The seeds obtained were sown in small pots in the autumn of 1952. Root-tips were then fixed and the chromosome numbers determined. During the winter the plants were kept in a cold frame and were planted out early next spring, at a distance sufficient to avoid competition between individuals. The chromosome numbers in the material (the five progenies taken together) were distributed in the following way (the number of accessories in brackets): (0) 237 plants, (1) 197 plants, and (2) 125 plants. The sum of plants being 559.

In the autumn they were cut down and weighed individually, excluding the root-system. The result is seen in Table 1. As there was no heterogeneity between progenies they were all put together.

Table 1.

Number of acc. chr.	Plant weight 0—100—200—300—400—500—600—700—800—900—1000—1100 gr.											n	$\bar{x}$ in gr.
0	7	13	33	56	44	42	20	12	4	6	—	237	439,45
1	3	14	23	36	47	29	27	9	4	5	—	197	457,62
2	2	6	12	28	16	29	16	12	2	1	1	125	480,40
	12	33	68	120	107	100	63	33	10	12	1	559	

As seen, there is a positive correlation between plant weight and number of accessory chromosomes. This correlation was tested by means of a covariance analysis and gave an r-value of 0,0827 with a t^2-value of 3,85 corresponding to a P of 0,05. The difference between the various progenies with respect to the average plant weight was rather small but no doubt increases the variation within chromosome numbers and in this way tends to lower the significance. When the subdivision of the material in progenies was considered and the joint correlation coefficient within progeny is calculated the result was an r-value= 0,0968 with a t^2-value of 5,22 corresponding to a P intermediate between 0,02 and 0,05.

From this result one may conclude that there is no negative effect of the accessory chromosomes on the plant weight in the present material. On the contrary there are indications of a positive effect.

Experiments in the greenhouse. — A series of similar experiments have been made in the greenhouse under more controlled conditions. The plants were grown in a greenhouse bed in sandy, calcerous soil carefully mixed. This type of soil is dominating in areas where accessory chromosomes are most frequent in Sweden.

The material used was the progeny of a single cross (20+1 acc.)×(20+1 acc.). The distribution of chromosome numbers (see Table 2) was about the same as for the material used in the field experiments. As all the plants are

siblings it is to be expected that the genotypic variation irrespective of accessories will be the same in all the groups of different chromosome numbers. Moreover, the modificative variation in this experiment should be smaller than in the earlier mentioned and small differences between groups thus easier to detect.

After germination in Petri dishes the seedlings were transplanted to the greenhouse bed. After about three months the plants were cut and weighed, root-tips were fixed and the chromosome numbers determined. The result is given in Table 2.

Table 2.

Number of acc. chr.	Plant weight 1,0–1,5–2,0–2,5–3,0–3,5–4,0–4,5–5,0–5,5–6,0–6,5–7,0–7,5–8,0 gr.														n	$\bar{x}$ in gr.
0	9	15	17	32	24	15	13	8	7	2	2	2	—	1	148	3,21
1	8	11	13	13	30	25	16	20	10	8	4	—	1	3	162	3,72
2	3	4	2	6	11	8	12	9	4	1	2	1	1	—	64	3,82
	20	30	33	51	65	48	41	37	21	11	8	3	2	4	374	

As seen, plants with one and two accessory chromosomes have an average plant weight of 3,72 gr. and 3,82 gr., whereas the corresponding figure for plants without accessory chromosomes is 3,21 gr. This difference was tested by means of an analysis of variance. The mean square for between and for within series is 52,13 and 6,98, respectively, which gives a $v^2 = 7{,}47$ corresponding to a P-value smaller than 0,001. For a more detailed analysis the sum of squares for "between" was divided into two parts. One corresponding to the variation between plants without accessory chromosomes and the sum of plants with accessory chromosomes. The other sum of squares gives the variation between plants with 1 and 2 accessory chromosomes.

Cause of variation	Degrees of freedom	Sum of squares	Mean square
Between plants without and the sum of plants with 1 and 2 accessories	1	102,3728	102,3728
Between 1 and 2 accessories	1	1,8785	1,7685
Within series	371	2589,2995	6,9792
	373	2693,5508	

When plants without accessory chromosomes are tested against plants with 1 and 2 the quotient obtained is 14,67 which gives a P-value smaller than 0,001. Plants with 1 accessory chromosome contrasted against plants with 2 accessory chromosomes gives a quotient of 3,72 with a P-value very close to 0,05 (the value 0,05 corresponds to the quotient 3,84). Thus there is a significant difference in plant weight between individuals without and with 1 and 2 accessory chromosomes. It is less certain whether there is a difference between plants with 1 and 2 accessory chromosomes, respectively.

This experiment demonstrates that 1 and 2 accessory chromosomes have a stimulating effect on vegetative development but this conclusion must be limited to the present material and the special environmental conditions during the experiment. It is also restricted to the early stage of development of the plants. As already mentioned, the plants were harvested already after three months to avoid competition between the plants. The problem of competition is certainly of interest but may be taken up in another connection.

Discussion and conclusions. — Summarizing the results of the experiments, it can be concluded that 1 and 2 accessory chromosomes in the present material and under the actual experimental conditions have a stimulating effect on the vegetative development of the plants. This effect, however, is fully clear only in the experiments carried out in the greenhouse. The significance of the results obtained in the field grown material is weak. The difference between the results of the experiments in the field and the greenhouse may depend on genotypic differences between the materials or on the fact that the environmental conditions in the greenhouse were more favourable for plants with accessory chromosomes. The difference may also be due to a stronger manifestation of the effect of the accessory chromosomes during the early stages of the vegetative development. The most probable explanation, however, is that the modificative variation in the field was bigger than in the greenhouse. This makes differences between chromosome numbers more difficult to detect.

As mentioned above, rather little has been published about the genetic effect of accessory chromosomes. The data available have been mainly obtained in rye (MÜNTZING, 1943) and maize (RANDOLPH, 1941). RANDOLPH studied the effect of accessory chromosomes on segregation of a number of genes in maize, but no influence was observed. On the other hand, it could be shown that a large number of accessory chromosomes had a detrimental effect on vitality and fertility. MÜNTZING studied the effect of the accessory chromosomes in rye on plant vigour and fertility and found a similar negative effect. With respect to the vegetative development this effect was marked only when higher numbers of accessory chromosomes were present whereas fertility was affected already by lower numbers. Also in *Centaurea* high numbers of accessory chromosomes have a negative effect on plant vigour (unpublished data). This is also demonstrated in natural populations where plants with higher numbers of accessory chromosomes are rare, although they could not only be produced in experimental crosses but were also easily maintained in culture.

The results in rye where even low numbers of accessory chromosomes have a deleterious effect in most respects are not inconsistent with those now presented for *Centaurea scabiosa*. One may assume that the response of a plant to accessory chromosomes depends on genotypic constitution as well as on external environmental conditions (*cf.* WHITE, 1951).

It is thus very probable that also in *Centaurea* a low number of accessory chromosomes is deleterious or neutral under certain conditions. The data on the geographical distribution (data by FRÖST in MÜNTZING, 1954) show that in certain regions accessory chromosomes are common and in others practically absent. These differences in frequency of accessory chromosomes are probably not accidental but correlated with certain edaphic and climatic factors.

It is not known whether the demonstrated effect of low numbers is limited to *Centaurea scabiosa*. Recently, BOSEMARK (unpublished data), however, has found indications of a similar positive effect of low numbers of accessory chromosomes in *Festuca pratensis*.

Institute of Genetics, University of Lund, Sweden.

Literature cited.

1. BOSEMARK, N. O. 1950. Accessory chromosomes in *Festuca pratensis* HUDS. — Hereditas XXXVI.
2. — 1954. On accessory chromosomes in *Festuca pratensis*. I. Cytological investigations. — Hereditas XL.
3. DARLINGTON, C. D. and UPCOTT, M. B. 1941. The activity of inert chromosomes in *Zea mays*. — Journ. of Genetics 41.
4. HÅKANSSON, A. 1948. Behaviour of accessory rye chromosomes in the embryo-sac. — Hereditas XXXIV.
5. MÜNTZING, A. 1943. Genetical effects of duplicated fragment chromosomes in rye. — Hereditas XXIX.
6. — 1944. Cytological studies of extra fragment chromosomes in rye. I. Iso-fragments produced by misdivision. — Hereditas XXX.
7. — 1945. Cytological studies of extra fragment chromosomes in rye. II. Transmission and multiplication of standard fragments and iso-fragments. — Hereditas XXXI.
8. — 1946. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at the pollen mitosis. — Hereditas XXXII.
9. — 1948. Cytological studies of extra fragment chromosomes in rye. IV. The position of various fragment types in somatic plates. — Hereditas XXXIV.
10. — 1954. Cytogenetics of accessory chromosomes (B-chromosomes). — Proc. IXth International Congress of Genetics (in the press).
11. MÜNTZING, A. and LIMA-DE-FARIA, A. 1949. Pachytene analysis of standard fragments and large iso-fragments in rye. — Hereditas XXXV.
12. — and — 1952. Pachytene analysis of a deficient accessory chromosome in rye. — Hereditas XXXVIII.
13. RANDOLPH, L. F. 1941. Genetic characteristics of the B chromosomes in maize. — Genetics 26.
14. ROMAN, H. 1947. Mitotic non-disjunction in the case of interchanges involving the B-type chromosome in maize. — Genetics 32.
15. TISCHLER, G. 1953. Allgemeine Pflanzenkaryologie, Ergänzungsband: Angewandte Pflanzenkaryologie.
16. WHITE, M. J. D. 1951. Cytogenetics of Orthopteroid Insects. — Advances in Genetics, Vol. IV.
17. ÖSTERGREN, G. 1947. Heterochromatic B-chromosomes in *Anthoxanthum*. — Hereditas XXXIII.

A. LIMA-DE-FARIA: The difference between a smear and a squash.

The two terms smear and squash are often used incorrectly as synonyms in cytological literature or are employed with rather vague meanings. The

difference between the two terms is not a simple academic one but they stand for two quite different methods which fulfil distinct ends in cytological technique.

The main differences between a smear and a squash are the following:

(1) A smear is normally made of a tissue composed of free cells or cells that are loosely held together. Smears are made, for instance, of blood tissue in animal cytology and of pollen mother cells or pollen grains in plant cytology. Squashes can be made of these same tissues as well as of other tissues in which cells are well kept together, such as tadpole tails, salivary glands, root tips, or leaf buds.

(2) The material used for a smear is living at the time the smear is made. The material used for a squash can be fixed and stored until it is used in the making of the preparation.

(3) A smear is made by spreading the cells of a tissue over the surface of a slide with the aid of another slide or a flat honed scalpel. In this way the cells, as well as the chromosomes, are separated from each other more than they were in the original tissue. In the squash the cells are pressed between cover slip and slide. This pressure over the cells is applied after the tissue has been made into very small pieces constituted by groups of a few cells.

(4) In a smear, once the material is spread over the slide, most fixing mixtures may be used. As the cells lie in a single layer and can be put into direct contact with the fixing mixture, the fixatives normally used are of the osmic and Navashin types, because these two kinds of fixatives, as a rule, give the best type of fixation. The formalin which hardens the tissues can be used, without reserve, because cells and chromosomes have been spread at the beginning of the procedure. In a squash, on the other hand, very few types of fixatives can be used if the material is going to be stored. The fixatives most commonly used are those of the acetic-alcohol type. If the squash is to be made at once, fixatives of the Navashin or Benda types may be used, but only for a short period of time. The material should not in any case become hard; otherwise the cells cannot be well spread when the pressure is applied.

(5) When the smear is made the exact meiotic stage cannot be checked easily and the spreading and staining of the chromosomes can only be observed at a later stage. When a squash is made the exact meiotic stage can be checked at once as well as the degree of staining and spreading of the chromosomes.

(6) The stains used in smears and squashes are usually different. As a rule, crystal violet and fuchsin-sulphurous acid are used for smears. They have the advantage of giving a clear contrast between the cytoplasm and the chromosomes. Iron-aceto-carmine, aceto-orcein and aceto-lacmoid are commonly used for squashes. These stains were introduced in cytology in connection with the development of the squash technique. They fix and stain simultaneously due to the 45 % acetic acid that they contain. However, such use of the different stains rests in part on tradition, because smears as well as squashes may be stained with all the stains just mentioned, although not to equal advantage.

(7) The smear was first used in animal histology primarily for the study of blood cells. It was introduced in plant cytology by TAYLOR in 1924 for the study of pollen mother cells. This technique had two big advantages over sec-

tions. It permitted a spreading of the cells and chromosomes, and it permitted at the same time a very quick and direct fixation of the chromosomes. The squash was introduced in plant cytology at about the same time (BELLING, 1921, 1926). It represented a further advance, because cells and chromosomes in plant tissues other than pollen mother cells and pollen grains, could be spread and thus more easily observed. At the same time the squash technique furnished a very rapid method of inspecting the meiotic and mitotic stages.

(8) One of the main advantages of the squash over the smear lies in the use of fixed material which can be stored for a few months. This permits the fixation of large amounts of material at a certain date — as during the flowering period of a species — and its use in preparations made at a later date. A second advantage lies in its application to nearly all of plant and animal tissues. Tissues which are too hard for a squash can be softened in several ways, or the cells can be made to separate more easily from each other. A third advantage is that the exact stage, and also the degree of spreading and staining of the chromosomes, can be much more easily watched when making a squash than when making a smear. On the other hand, a smear has several advantages over the squash. A better fixation can be easily secured because it is possible to place the cells quickly and in direct contact with the fixative. The difficulties of shrinkage and loss of cells during the procedure of making the preparations permanent are not so great in the case of a smear. Moreover, a good contrast between cytoplasm and chromosomes is more difficult to obtain with the stains normally used for making squashes than with those used for smears; fuchsin-sulphurous acid will result in good contrast in both cases.

A detailed description of a smear may be found in LA COUR (1937, p. 242 and 1947, p. 218) and a detailed description of a squash in LA COUR (1947, p. 224) and in LIMA-DE-FARIA (1948).

Institute of Genetics, University of Lund, Sweden.

Literature cited.

1. BELLING, J. 1921. On counting chromosomes in pollen-mother cells. — American Nat. 55: 573—574.
2. — 1926. The iron-acetocarmin method of fixing and staining chromosomes. — Biol. Bull. 50: 160—162.
3. LA COUR, L. 1937. Improvements in plant cytological technique. — Bot. Review 3 (5): 241—258.
4. — 1947. Improvements in plant cytological technique. II. — Bot. Review 13 (4): 216—240.
5. LIMA-DE-FARIA, A. 1948. B chromosomes of rye at pachytene. — Portug. Acta Biol. (A) 2 (3): 167—174.
6. TAYLOR, W. R. 1924. The smear method for plant cytology. — Bot. Gaz. 78: 236—238.

S. ELLERSTRÖM and A. HAGBERG: Competition between diploids and tetraploids in mixed rye populations.

When tetraploid and diploid rye have possibilities to intercross, triploid embryos are formed. Most of these embryos abort in an early embryonic stage

(HÅKANSSON and ELLERSTRÖM, 1950; MÜNTZING, 1951; LÖWENSTEIN, 1951). In practice this means a decreased seed setting often followed by a lowered yield of grain. Some artificial autotetraploids, *e. g.* rye, are now grown on a large scale. There is a risk that this tetraploid rye will be pollinated from adjacent fields of diploid rye and also that diploid seed will be mixed into the tetraploid. The first risk is easy to avoid by always keeping a sufficient distance between fields with diploid and tetraploid crops. The other risk is more insidious. So far, the only way to check if the tetraploid lots are mixed with diploid seed is to count the chromosome numbers in seedlings from random samples.

From a practical as well as from a theoretical point of view it is of interest to know what happens when different proportions of autotetraploid and diploid rye are mixed. In order to study this problem tetraploid rye was mixed with increasing percentages of diploids and sown in plots, 400 sq. m. each, separated from each other. One of these plots (I) was sown with carefully controlled pure tetraploid seed as a standard, the others were sown with the tetraploid seed mixed with diploids, as follows:

Plot	I	II	III	IV	V	VI	VII	VIII
Per cent of diploids	0	1/2	1	2	4	8	16	32

In addition, 20 well marked diploid plants were included in each of the plots II—VIII. These plants were continuously followed and the seed setting was examined at harvest time. Normally, diploid rye has a seed setting of 75—80 per cent; the tetraploid rye has 60—65 per cent, in plot I of the present experiment 63 per cent. In plot II the seed setting of the diploid plants was found to be 1,7 per cent and in plot VIII 31 per cent. On an average the tetraploid plants of plot VIII have a seed setting of 38 per cent, the lowest value found in tetraploid plants from the plots investigated. Thus, the frequency of diploids can be expected to decrease in this mixture because of higher fertility of the tetraploid.

It is of interest to study the vegetative competition. We were not able to examine individual plants of the populations but we estimated the proportions of diploid and tetraploid spikes by checking the chromosome numbers in 400 random spikes of plot VIII and 150 of plot VII. This analysis revealed 27 per cent diploid spikes instead of theoretically expected 32 per cent, and 9,3 per cent instead of 16 per cent, respectively. Thus, the diploid plants were also vegetatively suppressed by the tetraploids in the mixtures. This phenomenon will be studied more in detail in a repeated experiment.

The frequency of diploids in the plot progenies, *i. e.* the progenies from the original mixtures, proved for plot VIII to be 16,9 per cent or about half the frequency of germinable diploid seed mixed into the mother lot. The harvest of plot VII contained 2,3 per cent diploids instead of the original 16 per cent. Plot VI contained 0,6 per cent instead of 8. In the progenies of the other plots we did not find any diploids at all. Thus, the tetraploid rye shows a good ability to self-purification. This experience agrees with that gained from the commercial multiplication of tetraploid rye. It has proved rather easy to keep an elite of tetraploid rye free from diploids. This is definitely more difficult in

tetraploids of clover species. Especially the tetraploid alsike clover has proved very difficult to keep free from diploids but also tetraploid red clover has caused some trouble of this kind. *E. g.* a lot of commercial seed of tetraploid red clover containing 15 per cent of diploids was sown on two different farms. An analysis of the harvest of the two independent fields revealed that the proportion of diploids had increased to 65 and 75 per cent, respectively. There must be an equilibrium level in mixtures between diploids and tetraploids, where the frequencies may be unchanged through generations. In rye this equilibrium is at a diploid frequency higher than 32 per cent, but in red clover it is below 15 per cent.

It is interesting to study the correlation between the yield of grain and the different proportions of diploid and tetraploid rye in a mixture. The standard, plot I, yielded about 4000 kgs per hectare and plot VIII yielded 3400 kgs per hectare. The yield is lowered but not so much as we might expect with regard to the low seed setting. The yield of straw is especially high in plots with a high frequency of diploids. The relation between grain and straw yield is of some interest. Plot I had a grain/straw index of 0,74 and plot VIII had 0,59. The size of grain increases with increased frequency of diploids in the mixtures. Plot I gave a 1000-grain weight of 38 g and plot VIII gave 51 g. The low seed setting and the resulting low number of grains per unit area are partly compensated by the increased size of the grain.

The material further permits a comparison, in regard to the size of grain, between tetraploid plants and diploid plants with the same percentage of seed setting. At 35 per cent seed setting, the diploid plants gave an average 1000-grain weight of 38,3 g; the comparable value for tetraploid plants was 43,2 g. Since tetraploid rye has a lower seed setting than diploid, the difference in grain size is usually much greater. As a rule, tetraploid rye has about 50 per cent larger grains than diploid.

This abstract is a preliminary report from a study of artificial polyploids and their behaviour when commercially multiplied and grown as farm crops on a large scale. The data here presented are the results from only one year and must therefore be taken with caution.

The investigation has been facilitated by financial support from the Swedish Agricultural Research Council.

Svalöf in April, 1954.

Literature cited.

1. Håkansson, A. and Ellerström, S. 1950. Seed development after reciprocal crosses between diploid and tetraploid rye. — Hereditas XXXVI: 256—296.
2. Löwenstein, J. Prinz zu. 1951. Über die Befruchtungsverhältnisse zwischen diploidem und tetraploidem Roggen. — Zeitschr. Pfl.-z. 31: 104—133.
3. Müntzing, A. 1951. Cytogenetic properties and practical value of tetraploid rye. — Hereditas XXXVII: 17—84.

K. G. LÜNING: Maternal influence on a case of variegation in *Drosophila melanogaster*.

In some studies of induction of yellow mutations in Muller-5 chromosomes (*M5*, $sc^{S1}B$ *InS* w^a sc^8) (BONNIER 1954; LÜNING 1954) we obtained full mutants and mosaics. Among the mosaics there appeared three categories: first, those which were almost completely yellow but with a few grey bristles and hairs intermingled among the yellow ones — variegated yellow mutations; second, those with delimited grey and yellow areas with the latter including head, thorax or abdomen; and third, those with small yellow areas restricted to the thorax region. The latter group appeared in a rather high frequency — over 1 %. Their appearance was independent of irradiation of the *M5* males while the first two types appeared only after irradiation of the *M5* males. Further, the frequency of offspring with small yellow areas was lower with irradiated than with unirradiated mothers.

The latter observation suggested that a further study of individuals with small yellow areas would be interesting from the point of view of the possible causes determining the formation of these areas. At the beginning of the experiments two different explanations seemed possible, *viz.* first somatic crossing over, and second a variegation effect with low penetrance of the wild type yellow allele which lies close to a heterochromatic region.

The present paper deals with some preliminary experiments to narrow down the causes of the occurrence of these yellow areas referred to above before planning a more detailed investigation.

TABLE 1. *Virgin y w sn females were collected and stored 2 days. They were divided into two portions, one of which was irradiated without etherization with about 3000 r., and the other maintained as a control. Both portions were mated immediately after the irradiation to M5 males. Eggs were collected the 1st—3rd day and 8th—9th day after start of mating.*

Time from start of mating to egglaying	Irradiated females			Non-irradiated females		
	Total number of F_1 daughters examined	With yellow area		Total number of F_1 daughters examined	With yellow area	
		Abs. n:r	%		Abs. n:r	%
1—3	666	4	0,6	1843	64	3,5
8—9	817	39	3,6	868	30	3,5

In the experiments in which the small yellow areas were first detected we had used *y w sn* females mated to *M5* males. In the first test the same stocks were used but the *M5* males were not irradiated. The females were divided into two portions of which one was irradiated and the other not. The results are presented in Table 1. We see that irradiation of the females influences the frequency of yellow cases obtained together with a lessening of the effect as the time from irradiation to egglaying is increased.

TABLE 2. *y^{16} females mated to M5 males.*

Number of experiment	Total numbers of F_1 daughters examined	With yellow area	
		Abs. n:r	%
I	574	43	7,5
II	581	63	10,8
III	592	55	9,3
IV	1484	141	9,5
Total	3131	302	9,6

In order to determine if the appearance of the small yellow areas was a peculiarity of the *y w sn* stock, the experiment was repeated with another yellow stock derived from an irradiation experiment in this laboratory, and denoted y^{16}. This experiment has been repeated four times and the results are presented in Table 2. The data are striking in that the frequency of individuals with yellow bristles was much higher with the y^{16} females than in the preceding test with the *y w sn* females.

TABLE 3. *2 days old y^{16} females were irradiated with 3000 r and subsequently mated to M5 males. Eggs were collected in four 2-day periods.*

Time from irradiation to egglaying	Total number of F_1 daughters examined	With yellow area	
		Abs. n:r	%
1—2	1458	64	4,4
3—4	1448	85	5,9
5—6	1158	44	3,8
7—8	1156	42	3,6

There was also one experiment in which the y^{16} females were irradiated when 2 days old and immediately mated to *M5* males. These results are presented in Table 3. They differ from the data in Table 1 in that there is no decline in the frequency of individuals with yellow areas with increasing time between irradiation and egglaying. The cause of this difference has not been investigated further.

TABLE 4. *M5 females mated to y^{16} males.*

Total number of F_1 daughters examined	With yellow area	
	Abs. n:r	%
836	1	0,1

Parallel to these experiments the reciprocal cross was made with *M5* females mated to y^{16} males. The data are presented in Table 4. The failure of appearance of individuals with yellow areas at the same rate as in the preceding tests indicates that the cause of the formation of yellow areas can not be somatic crossing over as this phenomenon should be independent of the direction of the cross (STERN, 1936). Furthermore, it shows that there is a maternal effect on the appearance of yellow areas which, in turn, can be affected by irradiation. A maternal effect can be due to either autonomous or non-autonomous cytoplasmic factors. Before describing a test carried out to distinguish between these two alternatives, another experiment ought to be presented.

TABLE 5. y^{def} *females mated to M5 males.*

Total number of F_1 daughters examined	With yellow area	
	Abs. n:r	%
879	9	1

Since *y w sn* and y^{16} females when mated to *M5* males gave different frequencies of daughters with yellow areas, it seemed desirable to test for the effect with a yellow stock deficient for yellow locus. Such a stock viable in a homozygous condition was obtained some years ago from Dr. B. P. KAUFMANN. y^{def} females were mated to *M5* males. The results are presented in Table 5. The low frequency obtained is obviously different from those obtained when *y w sn* or y^{16} females were used.

TABLE 6. y^{16}/y^{def} *females derived from reciprocal crosses were mated to M5 males.*

Grandmother stock	Total number of F_1 daughters examined	With yellow area	
		Abs. n:r	%
y^{16}	858	5	0,6
y^{def}	830	9	1,1

Since a maximum difference exists between y^{16} and y^{def} stocks, it seemed most convenient to use them in a test of whether autonomous or non-autonomous plasmic factors are influential in producing the maternal effect referred to above. For that reason reciprocal crosses were made, *viz,* y^{16} ♀♀ × y^{def} ♂♂ and y^{def} ♀♀ × y^{16} ♂♂. Virgin F_1 daughters from each cross were mated to *M5* males. The results are presented in Table 6. As can be seen, the data from both series are about the same as that obtained from the y^{def} stock (Table 5). It indicates that the occurrence of yellow areas seems not to be caused by autonomous plasmic factors since the reciprocal crosses give the

same results. This suggests that the plasmic factors are gene controlled products. Further, the results were even more striking when one considers that the heterozygote y^{16}/y^{def} females gave the same result as the y^{def} stock. This could be due either to an effect of yellow locus or to modifying genes with a dominant effect in the y^{def} stock. This will be further studied in future experiments.

TABLE 7. *M5 females were irradiated with 3000 r and subsequently mated to y^{16} males. Eggs were collected the first three days after irradiation.*

Total number of F_1 daughters examined	With yellow area	
	Abs. n:r	%
866	0	0

As occurrences of individuals with yellow areas appeared to be determined by gene products from the mother, which could, in some manner, be inactivated by irradiation, it seemed possible that in *M5* females similar gene products, but originating from the wild type allele, could be inactivated by irradiation to give cases with yellow areas when mated to y^{16} males. To test this *M5* females were irradiated and mated to y^{16} males. Eggs were collected the first three days after irradiation. The results are presented in Table 7. As can be seen, there is no effect of irradiation of *M5* females in producing yellow areas.

Summary. — When Muller-5 males are mated to *y* females there are individuals among the offspring which show small yellow areas on the thorax. The frequency of these individuals is determined by the particular *y* stock used. These rates were shown to decrease when the females were irradiated. When the reciprocal cross was made there was a very low frequency of yellow areas — only one case was obtained. It is concluded that the occurrence of high rates of yellow areas can not be due to somatic crossing over. It is further shown that this maternal effect seems not to depend on autonomous plasmic factors.

Institute of Genetics, University of Stockholm.

Literature cited.

1. BONNIER, G. 1954. Secondary influences from X-ray irradiation on mutational processes in *Drosophila melanogaster*. — Heredity 8: 199—210.
2. LÜNING, K. G. 1954. Variations in the breakability of chromosomes in mature spermatozoa of *Drosophila melanogaster* at different modes of irradiation. — Heredity 8: 211—223.
3. STERN, C. 1936. Somatic crossing over and segregation in *Drosophila melanogaster*. — Genetics 21: 625—730.

H. O. T. SUOMALAINEN: The chromosome number of *Neomysis vulgaris* L. (Crustacea, Mysidae).

The Crustacea constitute one of the major animal groups, yet, despite their common occurrence, they have attracted the interest of the cytologists relatively little. The presented status of our knowledge of Crustacean chromosomes can be appreciated on the basis of MAKINO's (1951) »Atlas of the chromosome numbers in animals». In this work, the chromosome numbers of 156 Crustacean species are listed, which means that only little more than one half per cent of the known species have been submitted to cytological investigation.

As far as the author is aware, no information has been published concerning the chromosomes of the Mysidacea, a smallish order comprising about 300 species, and of a somewhat uncertain systematic position. As a first result of the author's investigations on the cytology of these shrimps, the spermatogonial chromosome number of *Neomysis vulgaris* is recorded in this paper.

The author's material of this species, which is common on the shores of the Baltic Sea, was collected on the SW coast of Finland at the Tvärminne Zoological Station (approx. 59° 50′ N lat., 23° 15′ E long.), at the end of July and at the beginning of August, 1952. At this time, the littoral swarms of the species consisted of males and females in a ratio of 1 to 12—15. The testes gave beautiful squash preparations by the use of the aceto-carmine method after pre-fixation in acetic alcohol (1:3 or 1:2) for 15 mins. They contained large numbers of spermatogonial cells in various phases of mitosis, but no signs of even the beginning of meiosis were recorded. The number of cells at different stages of mitotic prophase was especially high, thus indicating a slow progress of the spermatogonial divisions. Groups of cells at full metaphase were relatively rare. The metaphase plates were of the crowded type characteristic of most Crustacea. In the clearest cases 36 separate chromosome elements could be counted. The exact determination of the chromosome number is, however, rendered somewhat uncertain by the difficulty of distinguishing whether some »bipartite» elements really are two small chromosomes located near another, or one larger metacentric chromosome with a well accentuated primary constriction. If the former suggestion is correct, the number of chromosomes is $2n=36$ ($n=18$).

The knowledge of the chromosome numbers in the Mysidacea has a certain cytotaxonomic significance. From MAKINO's list it can be seen that the lower Crustacea commonly called Entomostraca have haploid chromosome numbers ranging from 4 to 21 (excluding certain older counts which indicate still lower numbers in two species of *Cyclops*). In the Isopoda, species occur with low chromosome numbers ($n=6$, 7 or 8), while most of them possess haploid numbers between 24 and 31, occasionally even ± 60. In the inadequately studied Amphipoda, certain species of *Gammarus*, for instance, have $n=13$, while other related forms have approximately the double number. Finally, in the Decapoda, the haploid numbers vary from 34 to 127, those between 52 and 64 being the most common ones. On the whole, it can thus be stated that in the Crustacea, the »type» chromosome numbers of the different orders are roughly correlated with the average size of the animals, and that the more primitive groups also generally have lower numbers than the more advanced

ones. Until more detailed investigations on the cytogenetics of Crustacea have been made, it cannot be decided whether this state of affairs may be regarded as evidence of polyploidy in the chromosomal evolution in this group.

If further investigations should prove that the type number of the Mysidacea is around 18 (haploid), this would certainly emphasize the special position of this group among the Malacostraca, also indicated by its other characteristics. The Mysidacea were formerly classified together with the Euphausiacea as Schizopoda, but the two orders are now believed to be distant and placed in different subclasses (or divisions) of the Malacostraca, *viz.* in Peracarida and Eucarida, respectively. An undetermined species of *Euphausidae*, investigated in 1909 by TAUBE (*cf.* MAKINO, *op. c.*, who places it erroneously among the Decapoda), has shown the diploid number 38. Of course, it is not advisable to give exaggerated significance to the resemblance in the chromosome numbers of these two groups, but it certainly fits with the hypothesis that in the evolution of the chromosome complements of the Crustacea, the usual trend has been from lower to higher numbers of chromosomes.

Department of Zoology, University of Turku, Finland.

Literature cited.

1. MAKINO, S. 1951. An atlas of the chromosome numbers in animals. — Iowa State College Press, 2nd ed., 290 pp.

TILL MEDARBETARNA.

Manuskript — helst *maskinskrivna* — torde insändas till Redaktionen (Adelgatan 3, Lund) i fullt tryckfärdigt skick. De böra vara *noga genomsedda* för undvikande av ändringar mot manuskriptet. Obs. kommateringen! Korrektionskostnaderna betalas av författaren. Korrektur ställes till Redaktionen. Direkt förbindelse mellan författaren och tryckeriet tillåtes icke.

Personnamn sättas med KAPITÄLER. De markeras i manuskriptet med en våglinje. Latinska namn på växter och djur samt ord och satser av särskild vikt *kursiveras* (enkel understrykning).

Figurer numreras med arabiska siffror. Figurförklaring (på avhandlingens språk) torde insändas *samtidigt* med illustrationsmaterialet.

Planscher numreras med romerska siffror och de i dem ingående bilderna med arabiska.

Tabeller åsättas arabiska siffror och förses med kort rubrik.

Citerade arbeten samlas i en litteraturförteckning. I texten hänvisas till denna genom angivande av författare och årtal. Har en författare utgivit flera publikationer under samma år, tilläggas efter årtalet små bokstäver (a, b, c, etc.). Samma beteckningssätt användes i litteraturlistan, vilken uppställes i alfabetisk ordning efter författarna och under dessa i kronologisk följd. Inga litteraturhänvisningar få göras genom fotnoter. Överhuvudtaget så få noter som möjligt!

Avhandlingarna skola vara skrivna på tyska, engelska eller franska. Det är önskvärt, att uppsatser på tyska och franska åtföljas av en resumé på engelska. Översättningar, som ombesörjas av Redaktionen, bekostas av författaren.

Åt varje författare lämnas 100 fria separat. Avhandlingar på ett ark och däröver förses gratis med särskilt omslag. Till ett pris av 15 kr. pr 100 st. lämnas, om så önskas, omslag till mindre uppsatser. Större antal särtryck fås till självkostnadspris. *Anteckning om eventuella extraseparat göres å första korrekturet.*

INNEHÅLL.

Sid.

MÜNTZING, ARNE, An analysis of hybrid vigour in tetraploid rye 265

LUNDQVIST, ARNE, Studies on self-sterility in rye, *Secale cereale* L. 278

LÜNING, K. G., Effect of oxygen on irradiated males and females of *Drosophila*.. 295

THERMAN, EEVA and TIMONEN, SAKARI, The prophase index and the occurrence of multipolar divisions in human cancer cells 313

HÅKANSSON, ARTUR, Meiosis and pollen mitosis in X-rayed and untreated spikelets of *Eleocharis palustris* 325

BOSEMARK, NILS OLOF, On accessory chromosomes in *Festuca pratensis*. I. Cytological investigations 346

NYGREN, AXEL, Investigations on North American *Calamagrostis*. I. 377

OLSSON, GÖSTA, Crosses within the *campestris* group of the genus *Brassica* 398

LIMA-DE-FARIA, A. and BOSE, S., Spectrophotometric analysis of aceto-carmine solutions 419

BOSEMARK, NILS OLOF, On accessory chromosomes in *Festuca pratensis*. II. Inheritance of the standard type of accessory chromosomes 425

ÅKERMAN, ÅKE and HAGBERG, ARNE, Intraspecific sterility in oats 438

HALL, OVE L., Hybridization of wheat and rye after embryo transplantation 453

MÜNTZING, ARNE, The cytological basis of polymorphism in *Poa alpina* 459

NILSSON, HERIBERT, Über hochkomplexe Bastardverbindungen in der Gattung *Salix* 517

ABSTRACTS.

HÅKANSSON, ARTUR, Transmission of accessory chromosomes in *Poa alpina* ... 523

OLDÉN, E. J., Giant pollen grains in fruit trees from colchicine treatment in vacuum 526

FRÖST, S., The genetic effect of accessory chromosomes in *Centaurea scabiosa*.. 529

LIMA-DE-FARIA, A., The difference between a smear and a squash 533

ELLERSTRÖM, S. and HAGBERG, A., Competition between diploids and tetraploids in mixed rye populations 535

LÜNING, K. G., Maternal influence on a case of variegation in *Drosophila melanogaster* 538

SUOMALAINEN, H. O. T., The chromosome number of *Neomysis vulgaris* L. (Crustacea, Mysidae) 542

Utgivet den 10 september 1954.